REMARKS

This amendment is submitted in response to the Office Action mailed January 2, 2008, in connection with the above-identified application (hereinafter, the "Office Action"). The Office Action provided a three-month shortened statutory period in which to respond, ending on April 2, 2008. Submitted herewith is a Petition for a One-Month Extension of Time extending the due date from April 2, 2008 to May 2, 2008. Accordingly, this amendment is timely submitted.

The Pending Claims

Claims 1-26, as amended, and new claim 27, remain pending. New claim 27 has been added to cover a preferred embodiment of the invention. Claims 4, 8-12 16 and 18-26 are withdrawn. Applicants understand that these claims will be rejoined and allowed once the pending claims are allowed. The claims have been amended as set forth herein, but no new matter is introduced by these amendments or by the presentation of the new claim. Therefore, they all should be entered at this time.

To correct a minor clerical error in claims 13, 14, and 16, the article "a" has been changed to the article "the." In claim 14, the term "expression" is inserted before the term "vector."

Applicants submit that the objections and rejections based on non-statutory subject matter, indefiniteness, lack of enablement and anticipation are overcome in light of the amendments and arguments presented in the response.

Accordingly, entry of these amendments is respectfully requested.

Rejection Under 35 U.S.C. §101

Claim 17 was rejected under 35 U.S.C. §101, as being directed to non-statutory subject matter. Applicants have amended the claim, which now recites "an isolated polynucleotide comprising SEQ ID NO:38." Withdrawal of the rejection is respectfully requested.

Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 1-2, 5-7 and 13-15 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants have amended claim 1 by (1) substituting the term "polynucleotide" (second occurrence) with the term "transcript;" (2) changing the plural form of "sequences" to its singular counterpart; (3) deleting the term "including; and (4) incorporating the phrases "wherein said 5' intronic sequence includes" before the phrase "an in-frame methionine codon." Support for these amendments can be found at paragraphs [0014], [0015], [0018], [0019], [0021], and [0041].

With regards to claim 15, the dependency of this claim has been changed to claim 13 instead of claim 14. In addition, the phrase "transfected with the expression vector" has been inserted before the phrase "according to." Support for this amendment can be found at paragraphs [0063], [0095], [0096] and the Examples.

In light of these amendments and remarks, Applicants submit that the rejection is overcome and should be withdrawn.

Rejections Under 35 U.S.C. §112, First Paragraph

Claims 1-3, 5-7, and 13-15 were rejected under 35 U.S.C. 112, first paragraph, for failure to comply with the enablement requirement. Applicants respectfully traverse the rejection.

At the outset, Applicants wish to point out that the absence of Jint-Jß2.1-Cß2 mRNA in an MBA-13 cell population is not an unexpected or inconsistent finding. This is because MBA-13 is not a cloned cell line. Instead, it is a cell strain that represents many cell types, which have been passaged in this manner for many cell generations. Therefore, long-term passaging of such cells causes selection of sub-populations, some of which may lack TCR. Further to the heterogeneity of the mesenchymal populations that may result in selection of one specific cell type following many passages, it should be kept in mind that mesenchymal (stromal) populations are highly plastic.

The plasticity of stem cells and mesenchymal cells is well known. In fact, one of the inventors, Dov Zipori, has addressed this issue in publications such as in "Cytokines Mol. Ther., 1996, 2(1):29-38, entitled "Control of Stroma-Dependent Hematopoiesis By Basic Fibroblast Growth Factor: Stromal Phenotypic Plasticity and Modified Myelopoietic Functions" (Appendix 1), and more recently, in the "Current Stem Cell Res. Ther., 2006, 1:95-102, entitled "The Stem State: Mesenchymal Plasticity As A Paradigm" (Appendix 2). Such plastic nature may account for variability in the behavior of cultured cells.

This, however, does not mean that primary cells isolated from the animal or human, when examined shortly thereafter, would be variable. To the contrary, such cells are relatively uniform. Indeed, most mesenchymal isolates, at first passages, are similar and one can, with great certainty, say that any normal mouse strain or a human individual will harbor TCR in the mesenchyme. This is certainly the case for fresh tissues.

In addition, Applicants respectfully submit that the presently claimed invention does not require undue experimentation. Thus, to use Jint-Jß2.1-Cß2 or any other TCR sequence as disclosed in the present application, a skilled artisan would only have to ascertain the presence and type of TCR transcripts using a simple polymerase chain reaction (PCR) screen of the extracted mRNA, which, by itself, cannot reasonably be considered as undue experimentation. PCR screening is a simple, relatively low cost, extremely sensitive and ultra-rapid procedure and well known to one skilled in the art. For example, a skilled artisan who wishes to analyze a TCR type in a mesenchymal tissue or cell can simply use PCR screening. The use of PCR screening is exemplified in Examples 2, 3 and 5 of the published patent application.

With respect to the Carroll reference (Cell, 2000, 101(6): 577-580), Applicants note that this reference fails to describe or suggest T cell receptors, in contrast to the claimed invention. Instead, the Carroll reference discusses the evolution of morphological diversity and, more specifically, the importance of cis-regulatory DNA and transcription factors in this phenomenon. Functional diversity or diversity at the protein level are not addressed. Moreover, there is nothing in the Carroll reference that suggests that similar genes do not share a similar function across species.

With respect to the Office Action's assertion that the disclosure of approximately 30 other TCR transcripts were derived from database hunting, Applicants note that most known sequences are probably found in data banks as products of large scale sequencing projects, such as the Human Genome Project or as expressed sequence tags (EST). As mentioned above, a simple PCR screen is all that is needed to ascertain which, if any, TCR transcript is found in the mesenchymal cells of interest.

In view of the above remarks, Applicants respectfully submit that the claimed invention, as set forth in claims 1-3, 5-7 and 13-15, is enabled. Accordingly, the rejection of these claims under 35 U.S.C. §112, first paragraph, should be withdrawn.

Rejections Under 35 U.S.C. §102(b)

Claims 1, 2, 6, 7, 13 and 14 have been rejected under 35 U.S.C. 102(e), as being anticipated by U.S. Patent Application Publication No. 20020138081 to Olga Bandman (hereinafter "Bandman"), as evidenced by Entrez Nucleotide Accession No. L34740 (http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=1100190), Entrez Nucleotide Accession No. AAA82787 (http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&id=1100191), and IGc domain description (http://www.ncbi.nlm.nih.gov/structure/cdd/cddsrv.cgi?uid=28981). In particular, the Office Action asserts that the nucleotides 1-139 of SEQ ID NO: 67 of the present application is identical to the nucleotides 35-173 of SEQ ID NO: 130 of Bandman (hereinafter "the Bandman sequence"). Therefore, the Bandman sequence anticipates the instant claims. Applicants respectfully traverse this rejection as explained herein.

At the outset, Applicants respectfully submit that the claimed invention, as set forth in amended claim 1, is drawn to an isolated polynucleotide that includes a transcript of a T cell receptor (TCR) gene that lacks V region sequences and comprises (1) a constant (C) domain; (2) a joining (J) region sequence; and (3) a 5' intronic J sequence that is upstream to the J region sequence, wherein the5' intronic J sequence includes an in-frame methionine codon.

Contrary to the claimed invention, the Bandman sequence is distinct from and not identical to the claimed polynucleotide transcript. At this juncture, Applicants would like to point out a possible typographical error in the description of the nucleotide assignment for the Bandman sequence. As provided in the Office Action at page 16, the following nucleotide assignment for the Bandman sequence is as follows:

> "Residues 1-124: genomic region 5' of the human Jß2.3 exon;
> Residues 125-324: Jß2.3 exon - intron - Jß2.4 exon;
> Residues 325-1642: exon 1 - exon 2 - intron B - exon 3 - exon 4 - of the 4 exon Cß2 domain;
> Residues 1643-1186: genomic region 3' of exon 4 of the Cß2 domain."

As underlined above, there cannot be a residue number 1642 in a 1186-bp long polynucleotide so that the reference may be to 1043 or 1143. Regardless of the typographical error and in view of

the alignment results, Applicants respectfully disagree with the Office Action's analysis of residues 325-1186.

By using the BLASTN sequence alignment program with residues 325-1186 of the Bandman sequence as Query and human germline TCR beta chain (accession number U66061) as Subject, enclosed herein as Appendix 3, it can be seen that residues 325-1186 of Bandman contain only the structure: exon 1 - exon 2 - intron B - exon 3 - exon 4 - of the Cß2 domain, without the genomic region 3' of exon 4. The Bandman sequence does not even contain the entire exon 4, since the last nucleotide aligns with base 204439 of U66061 (enclosed as Appendix 4), which is 12 nucleotides before the end of exon 4 of the Cß2 domain, situated at base 204451. Moreover, the Bandman sequence even seems to lack the poly A signal, situated between bases 204446-204451 of U66061 (see Appendix 4).

When the Bandman sequence is translated into a protein sequence using the first in frame methionine codon as the start codon, the first 46 amino acids, which encode the 5' intronic sequence upstream of Jß2.3 and the Jß2.3 sequence itself, are identical to the first 46 amino acids of SEQ ID NO: 51, which is the polypeptide encoded by SEQ ID NO: 67. However, the sequences diverge thereafter and there are several stop codons at positions 100, 140, 144, 173, 177, and 294, as shown in the attached Appendix 5.

Furthermore, a translation map of the Bandman sequence and its nucleotide assignment, as shown in Appendix 5, shows that the Bandman sequence comprises two different Jß2 sequences (Jß2.3 and Jß2.4) that are separated by an intron sequence. It also shows the presence of multiple stop codons, as mentioned above and underlined in Appendix 5, the first of which is placed at the start of the C region sequence. The presence of the stop codon at this particular position in the Bandman sequence would cause the sequence to be untranslatable.

Moreover, there is no indication in the Bandman application that SEQ ID NO:130 was successfully expressed in an expression system or transfected into a mammalian cell host, contrary to the claimed polynucleotide transcript.

The differences between the claimed polynucleotide transcript and the Bandman sequence are summarized in the scheme below:

<u>Claimed Polynucleotide transcript</u>: 5' intronic sequence- J - C

↓ Translation

Translated into a truncated TCR: 5' intronic sequence- Jβ2.3 - Cβ2

<u>Bandman's sequence</u>: 5' intronic sequence- Jβ2.3 -intron- Jβ2.4 - Cβ2

↓ Translation

Untranslatable

In addition, the present application recites "… a transcript of a TCR gene…lacking V region sequences and comprising a C domain and joining (J) region sequences and a 5' intronic J sequences upstream…including an in-frame methionine codon." The Bandman sequence has multiple J sequences separated by an intron. This is not equivalent to the claimed polynucleotide transcript or to its expressed protein.

Based on the above discussion, Applicants respectfully submit that the Bandman sequence is either an artifact or an immature product that has yet to undergo the recombination event that selects one J gene during TCR processing.

Furthermore, the present claimed invention is drawn to TCR transcripts specifically related to the mesenchyme and their use for modulation of mesenchymal cell growth or for detection of mesenchymal cells. Claim 27 specifically recites the mesenchymal (stromal) origin of the TCR transcript. This further distinguishes the invention from Bandman, which describes a plurality of cDNAs expressed in vascular tissue and their use for diagnosis, monitoring and treatment of vascular disorders.

Based on the foregoing remarks, Bandman fails to anticipate the subject matter as set forth in amended claim 1, as well as in dependent claims 2, 6, 7, 13 and 14. Accordingly, Applicants respectfully request the withdrawal of this rejection.

CONCLUSION

For at least the reasons set forth above, this application is in condition for allowance. Favorable consideration and prompt allowance of the claims are earnestly requested. Should the

Examiner have any questions that would facilitate further prosecution or allowance of this application, the Examiner is invited to contact the Applicants' representative designated below.

Respectfully submitted,

Date: 4/30/08

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APPENDIX 1

Control of stroma-dependent hematopoiesis by basic fibroblast growth factor: Stromal phenotypic plasticity and modified myelopoietic functions

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Abstract

It has been suggested that basic fibroblast growth factor (bFGF) affects hematopoietic cells directly and that it may also act indirectly by modulating stromal cell functions. We tested the response of phenotypically and functionally distinct stromal cell clones to this cytokine. We studied cell phenotype, the composition and organization of cytoskeleton and extracellular matrix, the ability to repopulate 'wounded areas', the expression of cytokine genes, and the capacity of the stroma to support long-term hematopoiesis in vitro. Although the impact of bFGF on cell growth was small, it induced a prominent morphological change in three stromal cell types that we tested. We analyzed the molecular basis for this change: bFGF modified the protein expression of α-smooth muscle actin (α-SMA), tropomyosin, α-tubulin, fibronectin and paxillin in a distinct manner characteristic of each of the stromal cell types. Immunofluorescence analysis of these proteins revealed profound changes in the cytoskeleton and extracellular matrix (ECM) networks accompanied by increased ability of the 14F1.1 stromal cells to scatter in in vitro 'wounded' areas. Furthermore, although only limited changes were monitored in the expression of cytokine genes, the ability of the stromal cells to support hematopoiesis was markedly modified. Thus bFGF profoundly changes the cellular organization of stromal cells, their adhesion and their motility properties. These changes are associated with modified capacity to support hematopoiesis in culture.

Key words:
bFGF; stroma; cytoskeleton; extracellular matrix; hematopoiesis

Introduction

bFGF, a member of the heparin-binding growth factor family, has been isolated from different sources, such as bone, kidney and brain, and has been implicated in embryonic development as well as in neovascularization, wound repair and hematopoiesis.[1–11] bFGF may affect hematopoiesis directly, as evidenced by its ability to induce the proliferation of purified human early hematopoietic stem cells[12] and its role in the differentiation of single human $CD34^+$, $HLA\text{-}DR^+$ and $CD38^-$ hematopoietic stem cells.[13] Although bFGF had no direct effect on committed hematopoietic progenitors, it synergized with granulocyte–macrophage colony-stimulating factor, erythropoietin and stem cell factor, to induce colony formation,[14,15] and nullified the inhibitory effect that TGF-β inflicted on human myeloid progenitor cells.[16]

Other effects of bFGF on hematopoiesis may probably be mediated indirectly by modification of stromal functions. The bone marrow stroma plays a crucial role in the regulation of hematopoiesis.[17–20] Changes in the cellular composition or function of stroma may result in altered hematopoietic activity. bFGF has been shown to be synthesized by stromal cells, and is a known stromal cell mitogen.[21–24] In human primary bone marrow cultures, addition of bFGF stimulated cell growth and increased cell density. Moreover, low concentrations of bFGF added to these cultures significantly augmented myelopoiesis.[10,25] This could be explained by increased cytokine secretion, since bFGF, as well as platelet-derived growth factor and tumor necrosis factor-α, induced colony-stimulating factor mRNA expression in TC-1 murine bone marrow stromal cells.[26]

Most studies of the effect of bFGF on stroma were performed using primary stromal cell populations composed of a variety of cell types. These different cellular components might react differently to bFGF. To address this possibility, we investigated the effect of bFGF on a number of cloned murine bone marrow-derived stromal cell lines. bFGF treatment resulted in changes in the expression of cytoskeletal and ECM proteins and in

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destruction of the cytoskeleton and ECM networks. These changes were accompanied by modified hematopoietic effects of the stromal cells.

Methods

Cell lines

To study the effect of bFGF on stromal cell functions, we utilized a series of stromal cell clones representing distinct subtypes of stromal cells.[27-28] These clones differ in their morphology, composition of ECM and capacity to support long-term hematopoiesis.[29] The following cell lines were used: 14F1.1 endothelial adipocytes, the MBA-15 osteogenic cell line and the MBA-13 fibroendothelial cell line.[27-32]

Cell cultures

Cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS) (BioLabs, Jerusalem). The cultures were incubated at 37°C in a humidified atmosphere of 10% CO_2 in air. One major advantage of the stromal cell lines derived in our laboratory[27-32] is their ability to be maintained at complete confluence for months of culture without detachment. Therefore we could apply bFGF to logarithmically growing cultures and maintain them by changing the medium once a week, along with supplementing fresh cytokine. These cultures were tested for expression of cytoskeletal, ECM protein, cytokine bioactivity and cytokine gene expression. After 3–4 weeks, confluent cultures, which were either treated with bFGF or control cultures, were seeded with bone marrow cells and studied for additional 2–3 week period. Long-term bone marrow cultures were supplemented with 10% horse serum (Biolabs, Jerusalem). Granulocyte–macrophage bone marrow colony assay was performed essentially as previously described.[33]

Antibodies and growth factors

The monoclonal antibodies (mAb) mouse anti-α-SMA, anti-α-tubulin and anti-tropomyosin (Bio-Makor, Rehovot, Israel) were used for immunoblots at optimal dilutions of 1:70, 1:500, 1:400 respectively. The mAb mouse anti-paxillin (Zymed, San Francisco, CA) was used at an optimal dilution of 1:4000 for immunoblots and 1:200 for immunofluorescence. The polyclonal rabbit anti-human fibronectin antiserum (Telios, San Diego, CA) was used at an optimal dilution of 1:2500 for immunoblots and at 1:500 for immunofluorescence. The antibodies applied in immunofluorescence studies were affinity-purified goat anti-mouse antibody conjugated to Texas Red (1:500) or rhodamine (1:30), and affinity-purified donkey anti-rabbit conjugated with FITC (1:100) (Jackson Immunoresearch Labs Inc, West Grove, PA). FITC-labeled phalloidin (1:50) was obtained from Molecular Probes Inc, and purified human bFGF was obtained from Pharmacia Biocenter, Nerviano, Milan.

Western blots

Stromal cells were seeded at $8 \times 10^3 - 2 \times 10^4$/ml in the presence or absence of bFGF for 3 days. Cells were then washed twice with cold phosphate buffer saline (PBS), removed by rubber policeman and lysed in TENN buffer (50 mM Tris, 5 mM EDTA, 150 mM NaCl, 0.5% Nonidet-40, pH 8.0) containing 1 mM phenylmethylsulfonyl fluoride (PMSF), and protein concentration was determined using the Bradford reagent (Biorad, Munich). Protein samples (25–50 μg/lane) and size markers (Bio-rad Laboratories, Richmond, CA) were run on 7.5% or 10% polyacrylamide gels and transferred to nitrocellulose paper. The ECL Western blotting kit (Amersham International plc, England) was used in all blots, with the procedure recommended in the kit. Blots were analyzed by a Molecular Dynamics (Model 300A) computing densitometer (Bio-rad Laboratories).

Immunofluorescence

Stromal cells were seeded at $8 \times 10^3 - 2 \times 10^4$ cells/ml on cover slips in the presence or absence of bFGF for 3 days. Alternatively, cells were seeded at the same concentrations and under the same conditions in chamber slides (Labtek slides, Miles Scientific, Naperville, IN) (400 μl/well). Cells were fixed in 3.7% paraformaldehyde in PBS for 20 min and permeabilized in 0.5% Triton X-100 in fixing solution for 2 min. Antibodies were applied as described by Burridge et al.[43] Stained samples were mounted in Entellan (Merck, Germany) or in 50% glycerol in PBS, and sealed with nail polish. The slides were examined using a Zeiss Photomicroscope III, equipped for epifluorescence observations, with an oil immersion plane neofluar objective, at a magnification of ×400.

Northern blots

Northern analysis was performed using the probes as detailed below. The DNA probes for cytokine genes and controls were obtained by restriction cutting from corresponding plasmids containing the genes as follows: rat TGF-β1, a 985 bp Hind III, BamHI fragment; mouse M-CSF, a 2.4 kb EcoRI fragment; mouse SCF, a 396 bp HindIII, XhoI fragment; IL-6, a 1.1 kb EcoRI fragment; bFGF, a 500 bp BamHI, NdeI fragment; and Activin A, a 345 bp NcoI, BstXI fragment. Actin was probed with a 3.5 kb HindIII fragment, and GAPDH with a 1.3 kb PstI, PstI fragment.

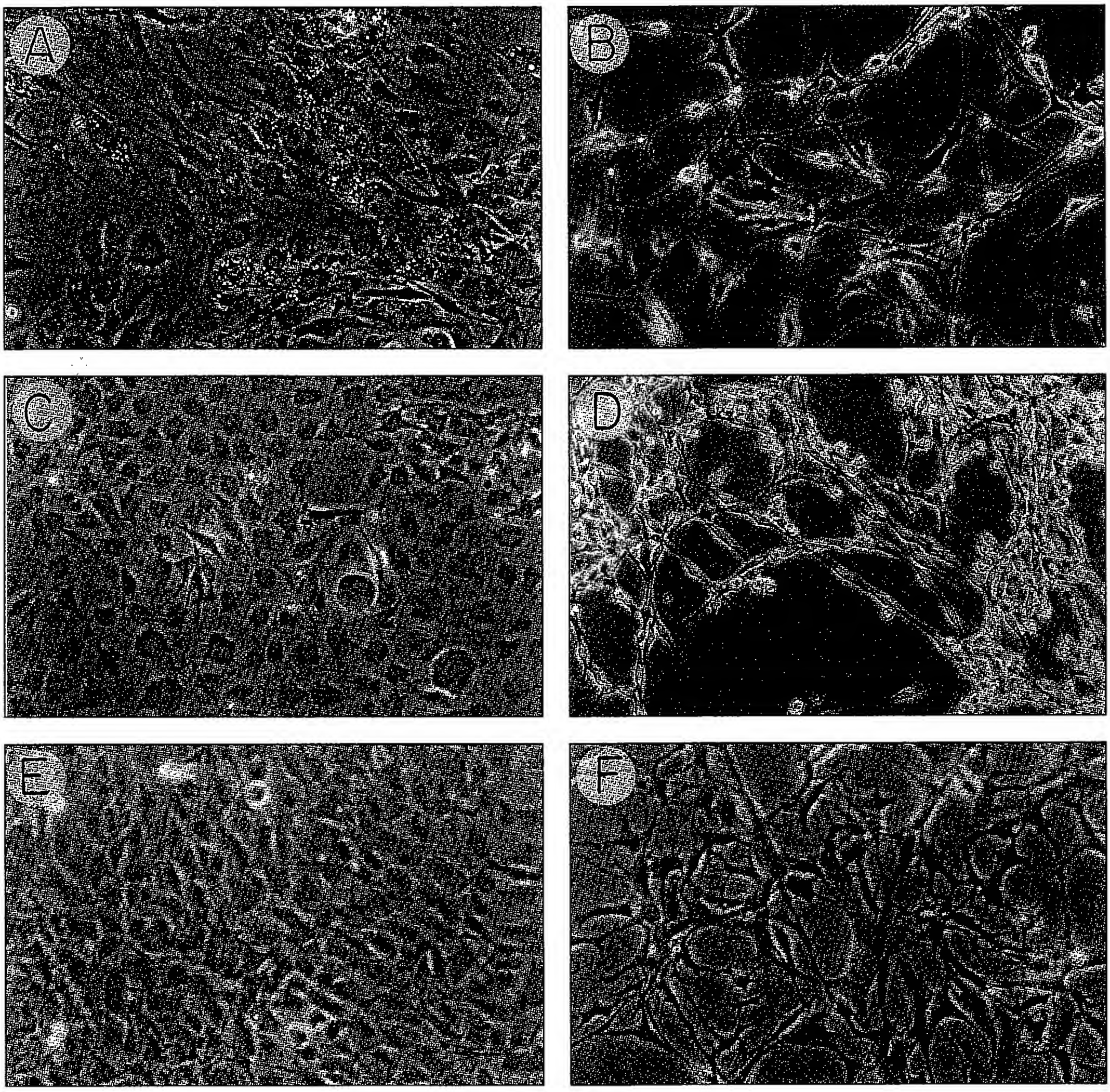

Figure 1
Morphological appearance of bone marrow stromal cells in the absence or presence of bFGF. 14F1.1 (A, B), MBA-15 (C, D) and MBA-13 (E, F) stromal cells were grown for 3–5 days in the absence (A, C, E) or presence of 10 or 20 ng/ml bFGF (B, D, F). Photomicrographs were taken in a phase microscope (Olympus CK2) at a magnification of ×100.

Results

Effect of bFGF on the expression and organization of cytoskeletal and ECM proteins of murine bone marrow-derived stromal cell lines

We used three different types of cloned stromal cell lines to study the effect of bFGF. Each of these types has distinct morphological and functional properties.[27–31] 14F1.1 endothelial-adipocytes were shown to support myelopoiesis and Pre-B lymphopoiesis,[29] MBA-15 cells are osteogenic cells,[32] and MBA-13 are fibroendothelial cells that promote the differentiation of Friend erythroleukemia cells.[30] The latter two stromal types do not induce hematopoiesis in culture. bFGF had a small effect on growth of the cell lines (not shown). On the other hand, it induced a dramatic morphological change in all three cell lines. In untreated cultures the cells characteristically displayed a flattened nonpolar shape (Figures

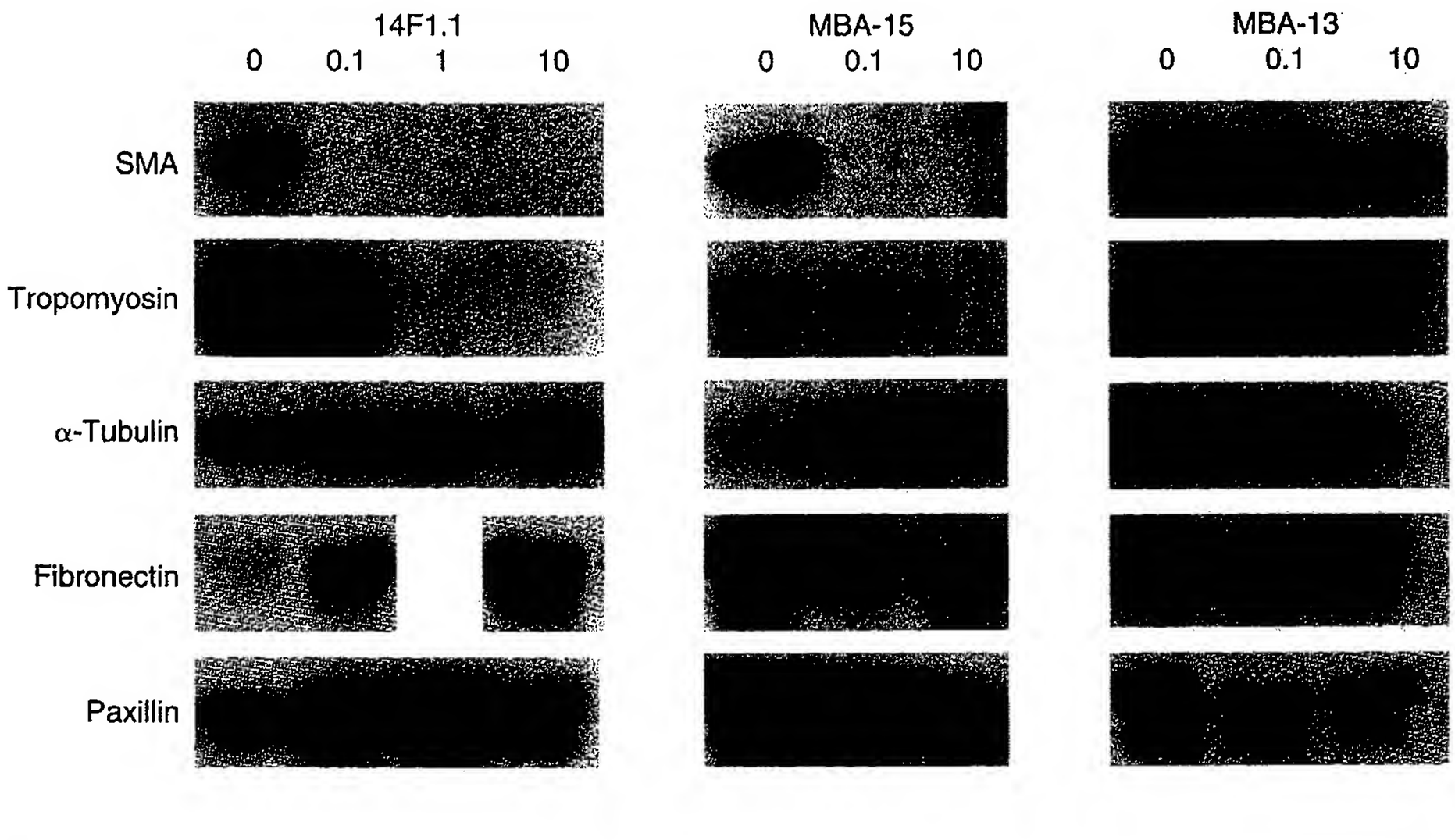

Figure 2
Western blot analysis of cytoskeletal and ECM proteins of bFGF-treated stromal cells. Stromal cells were grown in the absence or presence of bFGF (0.1, 1 or 10 ng/ml) for 3 days, washed twice with cold PBS and lysed with TENN buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% NP40 and 1 mM PMSF). Cellular protein extracts (25 μg/lane) were subjected to SDS-PAGE analysis under reducing conditions, along with size markers, and were then transferred to a nitrocellulose membrane. Immunoblots were subjected to ECL analysis followed by densitometric analysis.

1A, C and E), and, following addition of bFGF, the cells developed long cytoplasmic extensions: MBA-15 cells became spindle-like, while 14F1.1 and MBA-13 cells became dendritic-like with 14F1.1 cells being more strongly affected (Figures 1B, D and F).

The change in the stromal cells' shape led us to the assumption that the cytoskeleton and/or ECM of bFGF-treated cells were altered. A high proportion of actively proliferating 14F1.1 cells exhibit myofibroblast-like phenotype, characterized by flat, angulated appearance with α-SMA stress fibers.[34] Western blot analysis revealed that MBA-15 and MBA-13 cells also express α-SMA. Upon addition of bFGF (0.1–10 ng/ml), the expression of α-SMA was reduced in MBA-13 cells, and completely disappeared with addition of 0.1 ng/ml bFGF in 14F1.1 and MBA-15 cells (Figure 2). The expression of tropomyosin was also reduced in the three cells lines with addition of bFGF (Figure 2). In contrast to α-SMA and tropomyosin, the expression of the cytoskeletal protein α-tubulin was enhanced with bFGF treatment in 14F1.1 and MBA-15 cells (2- and 3-fold respectively) and was unchanged in MBA-13 cells (Figure 2). Treatment with 10 ng/ml bFGF resulted in an 8-fold increase in the expression of fibronectin in 14F1.1 cells, very little change in MBA-13 cells (1.4-fold increase) and an 80% reduction in MBA-15 cells (Figure 2). In agreement with these results, bFGF caused detachment of MBA-15 cells within 3 days in culture, whereas the adherence of 14F1.1 and MBA-13 cells was unaffected (not shown). Paxillin, a focal adherence protein,[35] increased 10-fold in its expression in response to 1 ng bFGF treatment in 14F1.1 cells. A 2-fold increase was observed in MBA-13 cells, and no change occurred in MBA-15 cells (Figure 2). Thus bFGF induced major, although different, changes in the expression of cytoskeletal proteins in each stromal cell line, suggesting that the cytoskeleton was differently organized in response to treatment with bFGF.

In order to examine this supposition, bFGF-treated 14F1.1 cells and corresponding controls were fixed and stained with phalloidin, anti-α-SMA, anti-fibronectin and anti-paxillin antibodies. bFGF treatment resulted in disappearance of actin stress fibers (Figure 3A versus B) and α-SMA fibers (Figure 3C versus D), which is in agreement with the results obtained from the Western blotting. Upon bFGF treatment, the organization of fibronectin (Figures 3E, F) and paxillin (Figures 3G, H) was disrupted, and neither the fibronectin fibers nor the focal contacts formed. In conclusion, bFGF not only induces changes in the expression of stromal proteins, but also destroys the organization of cytoskeleton and ECM networks.

The changes induced by bFGF in the cytoskeleton and the ECM were associated with modifications in the migratory functions of the stromal cells. Circular 'wounds' were made in the center of confluent cultures of the 14F1.1 cell line, and the cells were incubated for 4 days and then stained with an antibody to α-SMA. As can be seen in

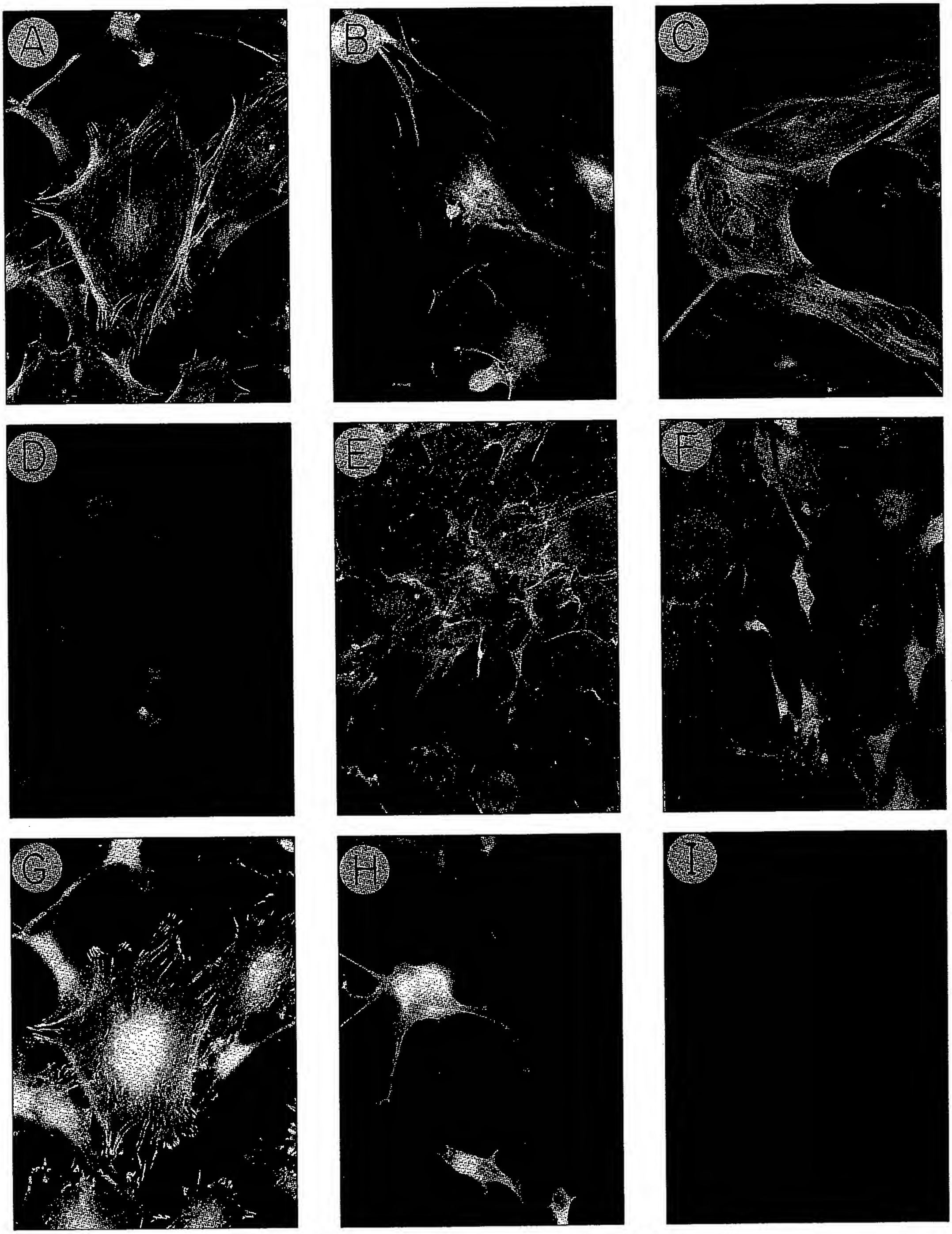

Figure 3
Immunofluorescent determination of cellular localization and organization of stromal cytoskeletal and ECM proteins following treatment with bFGF. 14F1.1 cells were grown on cover slips in the absence (A, C, E, G, I) or presence of 10 ng/ml bFGF (B, D, F, H) for 3 days, fixed with 3.7% paraformaldehyde, permeabilized with 0.5% triton in 3.7% paraformaldehyde, and stained with the relevant antibodies: FITC-labeled phalloidin (A, B), α-SMA (C, D), fibronectin (E, F), or paxillin (G, H). Fixed cells were then incubated with FITC or Texas-red conjugated second antibodies, washed and mounted in 50% glycerol in PBS. Background staining of cells subjected only to second

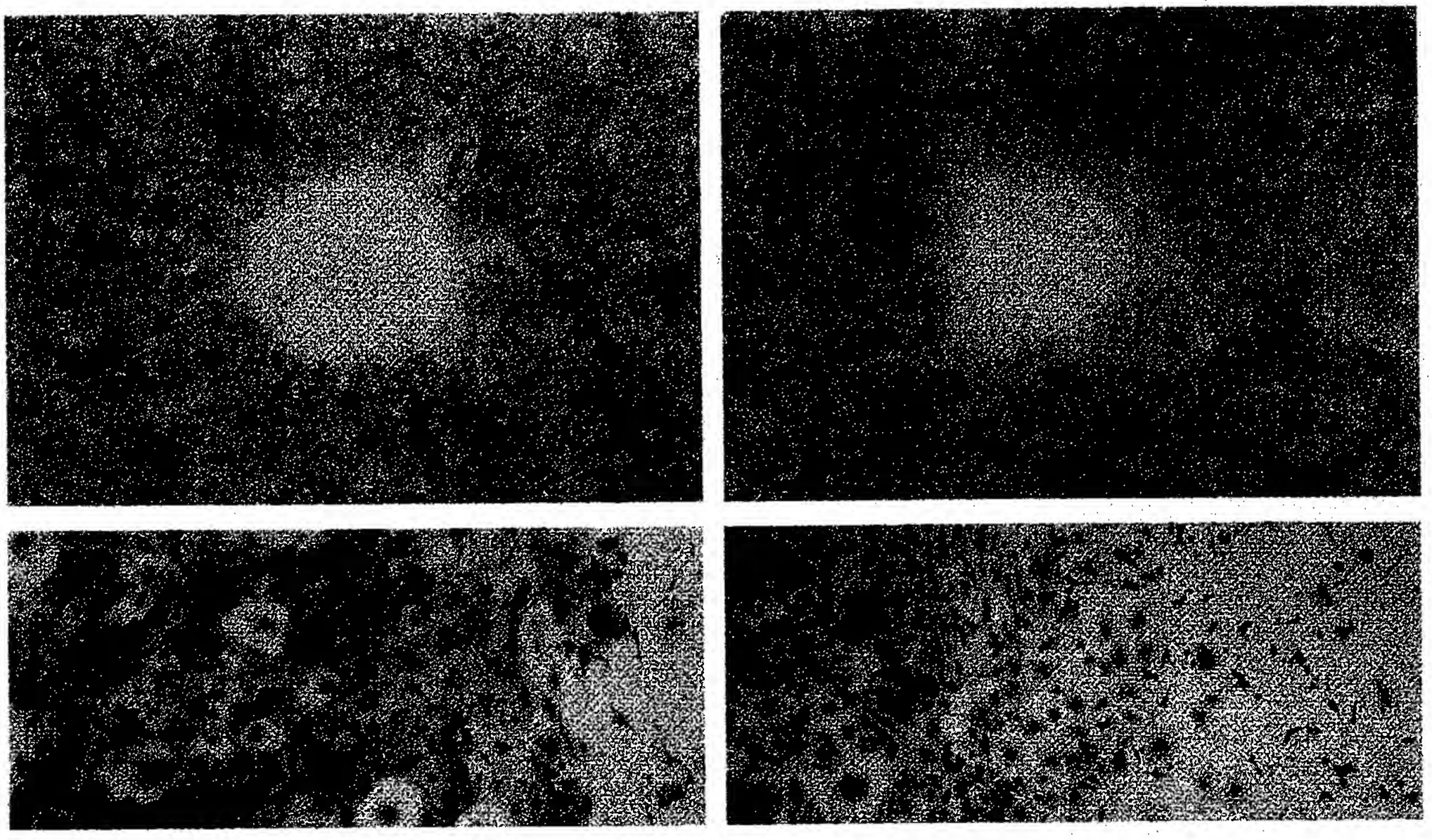

Figure 4
14F1.1 stromal cells were seeded in 35 mm tissue culture plates and were grown to confluence in the absence (left-hand side) or presence (right-hand side) of 10 ng/ml bFGF. Circular 6 mm 'wounds' were introduced. At 4 days culture, the plates were methanol-fixed and stained with α-SMA antibodies followed by biotinylated goat anti-mouse antibodies. The lower panels show a higher-magnification view of the 'wounds' shown in the upper panels.

Figure 4, the area adjacent to the wound contained a high concentration of α-SMA-positive cells, and the wound was closed slowly by gradual progress of its edges towards its center. By contrast, in the bFGF-treated cultures, α-SMA was undetectable, and the wound closed faster, as evidenced by the smaller diameter of the unoccupied area, due to migration of cells from the margins of the wound. The smaller size and polar morphology of the cells are characteristic of migration (Figure 4). The faster repopulation of the wounded area could not be attributed to increased proliferation of the stromal cells, since bFGF was found to be slightly inhibitory to the growth of 14F1.1 cells (not shown). In fact, studies with additional stromal cell lines show marginal growth stimulatory effect, and the overall effect of bFGF on the stroma is consistent with its being a morphogenic modulator[36] rather than a growth factor.

Effect of bFGF on cytokine expression and hematopoietic supportive activity of endothelial-adipose stromal cells

Changes in the properties of the stroma, such as those described above, could potentially lead to altered hematopoietic effects of the stroma. To test this possibility, we utilized the 14F1.1 stromal cell line. Previous studies showed that this cell line supports long-term myelopoiesis and lymphopoiesis in vitro,[29] whereas other stromal cell lines like MBA-13 and MBA-15 were incapable of performing similarly. The ability to release colony-stimulating factors into the growth medium was found to be inversely related to the capacity to support hematopoiesis.[37] Thus 14F1.1 was the poorest source of inducers. However, significant macrophage colony-stimulating factor (M-CSF), and transforming growth factor (TGF)-β titers were monitored in media conditioned by these cells (Peled et al, unpublished results). The expression of cytokine genes in stromal cells induced with bFGF was followed by Northern blotting using probes for M-CSF, TGF-β, bFGF, interleukin (IL)-6, activin A and stem cell factor (SCF) (Figure 5). In MBA-15 and MBA-13 cells IL-6 mRNA was markedly enhanced following triggering with bFGF. On the other hand, no such response was detected in 14F1.1 cells, which expressed very low levels of IL-6 transcripts. This is in agreement with previous observations indicating lack of IL-6 activity in media conditioned by the 14F1.1 stroma.[29] These cells responded to bFGF at 120 min following exposure with elevated levels of M-CSF mRNA, and at the same time point a dramatic increase in activin A transcripts was monitored (Figure 5). The elevated expression of both M-CSF and activin A genes was transient. Other cytokine

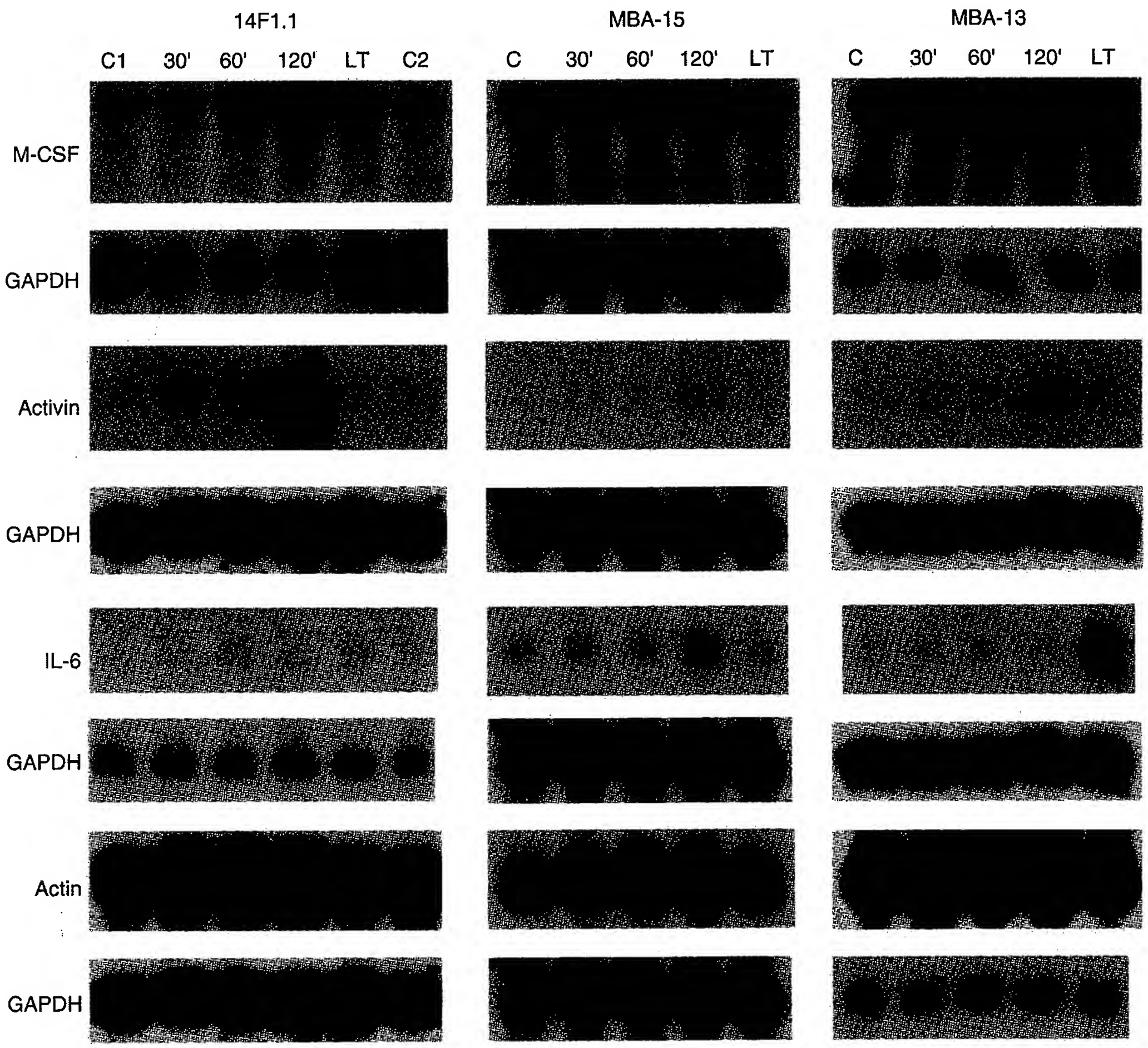

Figure 5
Northern blot analysis of cytokine genes. The expression of the genes encoding the indicated cytokines was examined following induction with bFGF and in control unstimulated cultures.

genes tested, namely TGF-β and bFGF, were found to be expressed by the different stromal clones, but did not show significant response to bFGF administration. SCF transcripts could not be detected in the stromal cell clones using Northern blotting.

The titer of stimulating activity released by the stroma was then monitored using a bone marrow colony assay. Addition of 0.01–10 ng of bFGF did cause a modest but statistically significant increase in the stimulating activity of the conditioned medium (Table 1). This may be expected from the fact that only transient changes were induced by bFGF in the expression of cytokine genes. In sharp contrast, when the bFGF-treated cells were tested for their ability to support long-term hematopoiesis, we found that low concentrations (particularly 0.5–1.0 ng/ml) were stimulatory for hematopoiesis, and increased dramatically the yield of GM-CFU recoverable from the cultures at week 2–3 incubation (Table 2). Relatively high concentrations of bFGF (10 ng/ml) were inhibitory to long-term maintenance of myeloid progenitors (Table 2).

Discussion

We describe here bFGF-induced phenotypic changes leading to alteration in hematopoietic functions of stromal cells. Changes in cell shape and in the patterns of expres-

Table 1
The effect of bFGF on secretion of colony-stimulating activities by 14F1.1 cells

bFGF (ng/ml)	Experiment 1	2	3
0	28 ± 4	77 ± 10	19 ± 3
0.01	29 ± 6	86 ± 17	31 ± 4
0.1	32 ± 7	114 ± 11	33 ± 11
0.5	56 ± 10*	113 ± 9	31 ± 6
1.0	55 ± 6*	123 ± 11	37 ± 1
5.0	41 ± 7*	104 ± 1	24 ± 7
10.0	38 ± 4*	113 ± 7	17 ± 6

14F1.1 stromal cells were grown in the presence or absence of bFGF for 3–4 weeks and then incubated for an additional week with or without the indicated concentration of bFGF. The conditioned medium was then collected and tested in a standard GM-CFC assay. The number of colonies per plate is indicated. Three different experiments were performed, and each point is the mean of triplicate plates ± SD. Statistical analysis was performed using Student's *t* test. Values marked by an asterisk are significantly different from the control value ($P < 0.05$).

Table 2
The effect of bFGF on stroma-dependent myelopoiesis

bFGF (ng/ml)	Experiment 1	2	3
0	141 ± 14	1120	5784 ± 396
0.1	95 ± 10	4700	ND
0.5	1053 ± 66	1364	ND
1.0	421 ± 59	2225	11700 ± 517
5.0	2985 ± 162	204	ND
10.0	70 ± 8	120	37 ± 16

The effect of bFGF on stromal cell-dependent myelopoiesis was tested using 14F1.1 stroma cells. The cells were pretreated with bFGF for 3 weeks. Bone marrow cells (10^5 cells/plate) were then seeded onto treated cultures of 14F1.1 cells. These co-cultures were maintained for 2–3 weeks in the presence or absence of bFGF. The cells found in the supernatant of the co-cultures were counted and examined in a GM-CFC assay. In experiments 1 and 3 each point represents the average number of myeloid progenitors/triplicate cultures, and in experiment 2 a single culture was tested.

sion of contractile proteins induced by bFGF and γ-interferon (γ-IFN) have been reported in other systems. In human bone marrow stromal cultures bFGF induced compact spindle-shaped morphology;[25] in bovine aortic endothelial cells it induced a more elongated phenotype accompanied by the loss of actin peripheral dense band.[38] In mouse embryo Swiss 3T3 fibroblasts bFGF caused rounded or bipolar morphology, lack of stress fibers and formation of small laemellipodia.[39] Similar effects to those induced by bFGF have been reported to be mediated by γ-IFN in fibroblastic populations.[40] In contrast, in fetal cardiac muscle cells an increase in skeletal α-actin and α-SMA, but not cardiac α-actin, gene expression occurred in response to bFGF treatment.[41] Thus a large body of evidence in the literature supports our findings regarding the inhibitory effects of bFGF on the polymerization and/or synthesis of actin stress fibers. It appears that bFGF causes changes in cellular morphology and in the cytoskeletal networks that are somewhat similar to those induced by colchicine, a microtubule-disrupting agent,[38] H-7 protein kinase inhibitor,[42] and herbimycin A, an inhibitor of tyrosine phosphorylation.[43]

In addition to the above, bFGF also induced different responses in each cell line in the production of the ECM protein fibronectin and the focal adherence protein paxillin. An increase in the production of both ECM-related proteins was evident in 14F1.1 cells. In Swiss 3T3 fibroblasts bFGF was shown to promote the expression of both tenascin and fibronectin ECM proteins.[39] Likewise, it induced an increase in tenascin levels in astrocytes,[44] and together with TGF-β1 had a synergistic effect on fibronectin secretion in cultured chicken chondrocytes.[45] Although in 14F1.1 cells the levels of the ECM-related proteins increased in response to bFGF, we have shown by immunocytochemistry that the overall organization of the proteins was destroyed, as was shown for the cytoskeletal stress fibers. Unlike the finding with 14F1.1 cells, fibronectin levels were reduced in MBA-15 and unchanged in MBA-13 in response to bFGF, and the levels of paxillin in both cell lines remained unchanged upon bFGF treatment. Thus our data demonstrate that each stromal cell line differed from the other types in its response to bFGF treatment. However, it should be noted that each set of changes induced by bFGF led eventually to the same final morpho-functional species, namely a polarized migratory-like cell.

The above results are consistent with a model of stromal cell phenotypic plasticity in which three distinct forms are involved. The functional steady-state form converts upon injury into a reactive proliferating cell (myofibroblast). Upon encounter with bFGF, the latter undergoes a cluster of gene expression changes that yield the third phenotype, namely the reparative (remodeling) fibroblast-like cell. These changes are relevant to increased morphogenetic changes, to repair and remodeling processes, and to pathological conditions such as myelofibrosis in which excessive stromal cell accumulation is associated with suppression of hematopoiesis.[46–47] As shown above, one consequence of continuous bFGF exposure of stromal cells is suppression of hematopoiesis.

The phenotypic changes induced by bFGF in stromal cells led to alterations in the capacity of the stroma to induce hematopoiesis. It is noteworthy that neither the increased hematopoietic activity of the bFGF-treated cultures nor the inhibitory effect may be explained on the basis of cytokine expression as summarized above. However, it cannot be excluded that cytokines other than those studied here contribute to the process. It is equally possible that the major impact on hematopoietic activity is that of the ECM, which, as shown in Figure 3, is dramatically affected by bFGF. In its altered form, the ECM functions differently in term of cytokine sequestration[48] and also in presenting adhesion sites for hematopoietic cells.[49] Since the process of stem cell adhesion to the stroma and the formation of cobblestone areas is dependent on ECM functions, its modification may well explain the observed altered hematopoietic activity of the stroma.

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References

1. Baird A, Esch F, Mormede P et al, Molecular characterization of fibroblast growth factor: distribution and biological activities in various tissues. *Recent Prog Horm Res* (1986) **42**: 143–203.
2. Rifkin DB, Moscatelli D, Recent developments in the cell biology of basic fibroblast growth factor. *J Cell Biol* (1989) **109**: 1–6.
3. Tamm I, Kikuchi T, Zychlinsky A, Acidic and basic fibroblast growth factors are survival factors with distinctive activity in quiescent BALB/c 3T3 murine fibroblasts. *Proc Natl Acad Sci USA* (1991) **88**: 3372–6.
4. Burgess WH, Masiag T, The heparin-binding (fibroblast) growth factor family of proteins. *Annu Rev Biochem* (1989) **58**: 575–606.
5. Rieck P, Assouline M, Hartmann C, Pouliquen Y et al, Effect of recombinant human basic fibroblast growth factor (rh-bFGF) on wound healing of the corneal epithelium. *Ophthalmologe* (1993) **90**: 646–51.
6. Lees VC, Fan TP, A freeze-injured skin graft model for the quantitative study of basic fibroblast-growth factor and other promoters of angiogenesis in wound healing. *Br J Plast Surg* (1994) **47**: 349–59.
7. Norioka K, Mitaka T, Mochizuki Y, Hara M et al, Interaction of interleukin-1 and interferon-gamma on fibroblast growth factor induced angiogenesis. *Jpn J Cancer Res* (1994) **85**: 522–9.
8. Soubrane G, Cohen SY, Delayre T, Tassin J et al, Basic fibroblast growth factor experimentally induced choroidal angiogenesis in the minipig. *Curr Eye Res* (1994) **13**: 183–95.
9. Pepper MS, Wasi S, Ferrara N, Orci L et al, In vitro angiogenic and proteolytic properties of bovine lymphatic endothelial cells. *Exp Cell Res* (1994) **210**: 298–305.
10. Wilson LE, Rifkin D, Kelly F, Hannocks M-J et al, Basic fibroblast growth factor stimulates myelopoiesis in long-term human bone marrow cultures. *Blood* (1991) **77**: 95–60.
11. Bikfalvi A, Han ZC, Angiogenic factors are hematopoietic growth factors and vice versa. *Leukemia* (1994) **8**: 523–9.
12. Gabbiannelli M, Sargiacomo M, Pelosi E, Testa U et al, 'Pure' human hemopoietic progenitors: permissive action of basic fibroblast growth factor. *Science* (1990) **249**: 1561–4.
13. Huang S, Terstappen LW, Lymphoid and myeloid differentiation of single human CD34+, HLA-DR+, CD38– hematopoietic stem cells. *Blood* (1994) **83**: 1515–26.
14. Gallicchio VS, Huges NK, Hulette BC, DellaPuca R et al, Basic fibroblast growth factor (B-FGF) induces early- (CFU-s) and late-stage hemopoietic progenitor cell colony formation (CFU-gm, CFU-meg, and BFU-e) by synergizing with GM-CSF, Meg-CSF, and erythropoietin, and is a radioprotective agent in vitro. *Int J Cell Cloning* (1991) **9**: 220–32.
15. Gabrilove JL, White K, Rahman Z, Wilson LE, Stem cell factor and basic fibroblast growth factor are synergistic in augmenting committed myeloid progenitor cell growth. *Blood* (1994) **83**: 907–10.
16. Gabrilove JL, Wong G, Bollenbacher E, White K et al, Basic fibroblast growth factor counteracts the suppressive effects of transforming growth factor-β1 on human myeloid progenitor cells. *Blood* (1993) **81**: 909–15.
17. Zipori D, Stromal cell lines from mouse bone marrow: a model system for the study of the hemopoietic microenvironment. In: *Experimental hematology today* (ed S Baum, D Pluznik, LA Rozenszajn) 55–63. Springer-Verlag, New York, 1985.
18. Dexter TM, Stromal cell associated haemopoiesis. *J Cell Physiol Suppl* (1982) **1**: 87–94.
19. Zipori D, The renewal and differentiation of hemopoietic stem cells. *FASEB J* (1992) **6**: 2691–7.
20. Deryugina EI, Muller-Sieburg CE, Stromal cells in long-term cultures: keys to the elucidation of hematopoietic development? *Crit Rev Immunol* (1993) **13**: 115–50.

21. Brunner G, Nguyen J, Rifkin DB, Wilson LE, Basic fibroblast growth factor expression in human bone marrow and peripheral blood cells. *Blood* (1993) **81**: 631–8.
22. Brunner G, Metz CN, Nguyen H, Gabrilove J et al, An endogenous glycosylphosphatidylinositol-specific phospholipase D releases basic fibroblast growth factor–heparan sulfate proteoglycan complexes from human bone marrow cultures. *Blood* (1994) **83**: 2115–25.
23. Thomson BM, Dean V, Farquharson C, Noble BS, Visualisation and selective elimination of a subpopulation of mitogen-responsive bone marrow stromal cells using bromodeoxyuridine and photo-induced cell killing. *Bone* (1993) **14**: 779–86.
24. Nishikawa S, Nakasato M, Takakura N, Ogawa M et al, Stromal cell-dependent bone marrow culture with a nearly protein-free defined medium. *Immunol Lett* (1994) **40**: 163–9.
25. Oliver LJ, Rifkin DB, Gabrilove J, Hannocks MJ et al, Long-term culture of human bone marrow stromal cells in the presence of basic fibroblast growth factor. *Growth Factors* (1990) **3**: 231–6.
26. Abboud SL, Pinzani M, Peptide growth factors stimulate macrophage colony-stimulating factor in murine stromal cells. *Blood* (1991) **78**: 103–9.
27. Zipori D, Friedman A, Tamir M, Silverberg D et al, Cultured mouse marrow cell lines: interactions between fibroblastoid cells and monocytes. *J Cell Physiol* (1984) **118**: 143–52.
28. Zipori D, Duksin D, Tamir M, Argaman A et al, Cultured mouse marrow stromal cell lines II. Distinct subtypes differing in morphology, collagen types, myelopoietic factors, and leukemic cell growth modulating activities. *J Cell Physiol* (1985) **122**: 81–90.
29. Zipori D, Lee F, Introduction of interleukin-3 gene into stromal cells from the bone marrow alters hemopoietic differentiation but does not modify stem cell renewal. *Blood* (1988) **71**: 586–96.
30. Zipori D, Toledo J, von der Mark K, Phenotypic heterogeneity among stromal cell lines from mouse bone marrow disclosed in their extracellular matrix composition and interactions with normal and leukemic cells. *Blood* (1985) **66**: 447–55.
31. Zipori D, Cultured stromal cell lines from hemopoietic tissues. In: *Handbook of the hemopoietic microenvironment* (ed M Tavassoli) 287–333. The Human Press, Inc, Clifton, NJ, 1989.
32. Benayahu D, Kletter D, Zipori D, Weintroub S, Bone marrow-derived stromal cell line expressing osteoblastic phenotype in vitro and osteogenic capacity in vivo. *J Cell Physiol* (1989) **140**: 1–7.
33. Freshney MG, Colony-forming assays for CFC-GM, BFU-E, CFC-GEMM, and CFC-mix. In: *Culture of hematopoietic cells* (ed RI Freshney, IB Pragnell, MG Freshney) 265–8. Wiley–Liss, New York, 1994.
34. Peled A, Zipori D, Abramski O, Ovadia H et al, Expression of α-smooth muscle actin in murine bone marrow stromal cells. *Blood* (1991) **78**: 304–9.
35. Turner CE, Glenney JR, Burridge K, Paxillin: a new vinculin-binding protein present in focal adhesion. *J Cell Biol* (1990) **111**: 1059–68.
36. Smith JC, Mesoderm-inducing factors in early vertebrate development. *EMBO J* (1993) **12**: 4463–70.
37. Peled A, Zipori D, A comparative study of growth stmulating and inhibiting activities secreted by a series of stromal cell lines. In: *Molecular biology of haematopoiesis* (ed NG Abraham, G Konwalinka, P Marks et al) Vol 2: 247–56 Intercept, Andover, 1992.
38. Liaw L, Schwartz SM, Microtubule disruption stimulates DNA synthesis in bovine endothelial cells and potentiates cellular response to basic fibroblast growth factor. *Am J Pathol* (1993) **143**: 937–48.
39. Tucker RP, Hammarback JA, Jenrath DA, Mackie EJ et al, Tenascin expression in the mouse: in situ localization and induction in vitro by bFGF. *J Cell Sci* (1993) **104**: 69–76.
40. Desmouliere A, Rubbia-Brandt L, Abdiu A, Walz T et al, α-Smooth muscle actin is expressed in a subpopulation of cultured and cloned fibroblasts and is modulated by γ-interferon. *Exp Cell Res* (1992) **201**: 64–73.
41. Parker TG, Chow K-L, Scwartz RJ, Schneider MD, Differential regulation of skeletal α-actin transcription in cardiac muscle by two fibroblast growth factors. *Proc Nat Acad Sci USA* (1990) **87**: 7066–70.
42. Volberg T, Geiger B, Citi S, Bershadsky AD, The effect of protein kinase inhibitor H-7 on the contractility, integrity and membrane anchorage of the microfilament system. *Cell Motil Cytoskeleton* (1994) **29**: 321–38.
43. Burridge K, Turner CE, Romer L, Tyrosine phosphorylation of paxillin and pp125^{FAK} accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. *J Cell Biol* (1992) **119**: 893–03.
44. Meiners S, Marone M, Rittenhouse JL, Geller HM, Regulation of astrocytic tenascin by basic fibroblast growth factor. *Dev Biol* (1993) **160**: 480–93.
45. Wu LNY, Genge BR, Ishikawa Y, Wuthier RE, Modulation of cultured chicken chondrocytes by transforming growth factor-β and basic fibroblast growth factor. *J Cell Biochem* (1992) **49**: 181–98.
46. McCarty D, Fibrosis of the BM: content and causes. *Br J Haematol* (1985) **59**: 1–7.
47. Biagini G, Severi B, Govoni E, Preda P et al, Stromal cells in primary myelofibrosis: ultrastructural observations. *Virchows Arch* [B] (1985) **48**: 1.
48. Vlodavsky I, Bar-Shavit R, Korner G, Fuks Z, Extracellular matrix-bound growth factors, enzymes, and plasma proteins. In: *Molecular and cellular aspects of basement membranes* (ed DH Rohrbach, R Timpl) 327. Academic Press, San Diego, 1993.
49. Teixido J, Hemler ME, Greenberger JS, Anklesaria P, Role of b1 and b2 integrins in the adhesion of human CD34 stem cells in the bone marrow stroma. *J Clin Invest* (1992) **90**: 358–67.

APPENDIX 2

The stem state: Mesenchymal plasticity as a paradigm

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Abstract

The mesenchyme is a remarkably plastic tissue in the embryo. Recent studies led to the discovery of mesenchymal cells in the adult organism that can differentiate in vitro into unexpected directions, beyond the well known ability of the mesenchyme to give rise to mesodermal derivatives. These studies highlighted the plastic nature of the mesenchyme, also beyond the embryonic developmental stage. This review discusses the possible functions of the mesenchyme in the adult and the reason for the maintenance of plasticity throughout mammalian life. The properties of the mesenchymal cells clearly exemplify the stem state concept; cells whether early or late in the differentiation cascade may assume a stem state that entails high plasticity.

Key words: stem cells, stem state, mesenchyme, plasticity

Plasticity of the embryonic mesenchyme

The development of the mammalian embryo provides the ultimate example of the plastic nature of cellular phenotypes. Cells shift from an epithelial to a mesenchymal phenotype in a sequential manner. This process called epithelial mesenchymal transition (EMT), also occurs in a reciprocal manner, thus, mesenchymal cells assume an epithelial phenotype, a process designated mesenchymal epithelial transition (MET). These extreme fluctuations in the nature of the cell entail changes in cell-to-cell adhesion, modification of the extracellular matrix and of cell motility, to point out just a few features that underlie these processes, based on marked changes in gene expression (reviewed in [1]). The notion that embryonic mesenchyme has a multipotent nature gained support from the isolation of C3HT101/2 cells. The latter is a stable cell line that originated from embryonic tissues. It is multipotent in that it gives rise to several types of mesodermal derivatives upon induction with agents such as 5-azacytidine [2] and differentiates more specifically upon induction with bone morphogenic protein-2 [3] or transforming growth factor β1 [4]. Therefore, this cell line is regarded as a mesenchymal stem cell (MSC). The C3HT101/2 cell line seems to have physiological counterparts since embryonic stem cells are found in maternal blood [5,6] and thus seem to be present in the embryo and to have migratory properties. A recent report suggests that MSCs are localized during development in the hemopoietic organs and spread from this locality to other sites in the adult [7]. The remarkable plasticity of embryonic mesenchyme was recently demonstrated by the isolation of primary cells from embryonic somatic explants. The cells were termed fetal somatic stem cells (FSSC) and were shown to integrate into embryonic tissues flowing injection into the blastocyst. The donor cells were found in the tongue, liver and muscle and some of them may have differentiated into lymphoid cells, however, no precise determination of the nature of the cells that arise from FSSC was reported [8]. Thus, plastic behavior is not foreign to mammalian cells and is well exemplified by embryonic mesenchyme. This plastic property is assumed to be a trait specific to embryonic cells that is lost in the adult, although it may be regained upon tumorigenic transformation and acquisition of metastatic properties (reviewed in [1]). In the review below, I shall discuss some past data as well as recent observations that support the notion of plasticity of the mesenchyme in the adult organism. This plastic behavior is, as I recently argued, the hallmark of the stem state [9,10].

Early indications for the plastic nature of adult mesenchyme: Friedenstien's bone marrow stroma that possesses the capacity to recreate in vivo ectopic hemopoietic microenvironment

Studies performed in the Soviet Union by AJ Friedenstein in the early 1970's have indicated that within the bone marrow reside cells capable of forming fibroblastoid colonies in vitro [11,12]. It was assumed, on grounds of histological analysis of the bone marrow, that these cells are derived from the mesenchymal stroma that forms the

supportive anlage of blood-forming tissues. By the same token, similar mesenchymal elements were supposed to form the supportive anlagen of any tissue and organ. Are all tissue stromal cells the same or does each organ contain a different type of stroma that create a specific microenvironment? To examine this issue Friedenstein attempted transplantation of the cultured mesenchyme under the kidney capsule. The cultured fibroblasts induced the formation of a bony structure at the site of implantation, and within this bone structure hemopoiesis occurred [13] (Figure 1). It was shown that the bone structure is of donor origin while hemopoietic cells encapsulated within the bone structure were of host origin, thus, the conclusion drawn by Friedenstein was that the cultured fibroblasts are osteogenic and, in addition, these cells carry the information necessary for the establishment of a hemopoietic inductive microenvironment (reviewed in [14]. Thus, these very early studies have already highlighted the width of activities of the bone marrow mesenchyme: osteoblastic capacity along with ability to dictate the differentiation of hemopoietic stem cells. As to the original question, whether the bone marrow stroma is specific to this organ; to date this question has remained unanswered since one can derive osteogenic mesenchyme that supports hemopoiesis from other tissues and organs. Whether the organ mesenchymal stroma in situ has specific properties that distinguishes one organ stroma from the other, should await genomic and proteomic analysis of cells within their natural habitat, rather than the study of cells isolated and propagated in vitro.

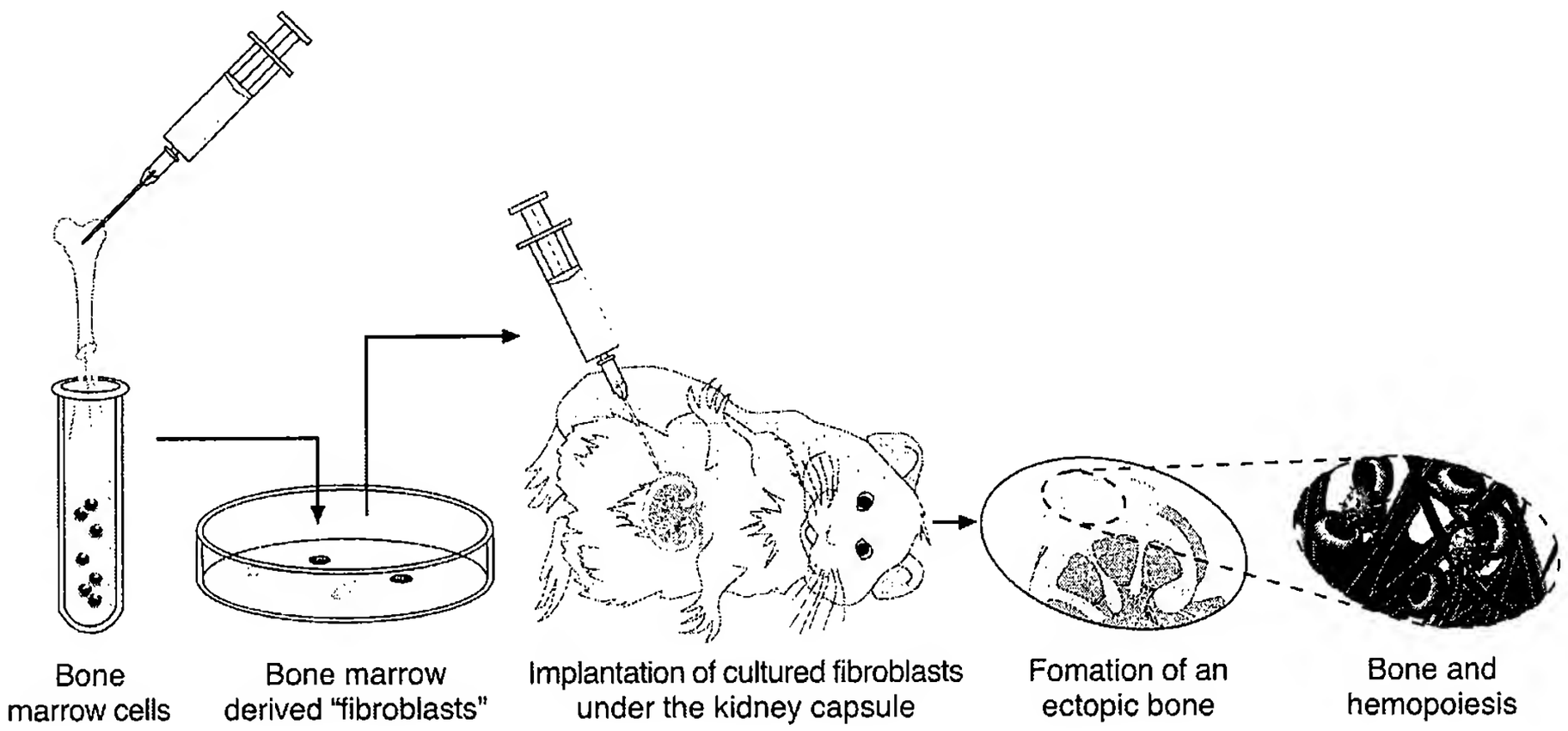

Figure 1: Friedenstein's ectopic osteogenesis and hemopoiesis model- Bone marrow cells are isolated and seeded in vitro in simple medium. This results in elimination of most of the hemopoietic cells and bone marrow derived fibroblasts survive. These cells are implanted under the kidney capsule where they form a bony structure that is colonized by hemopoietic cells from the host.

Dexter's long-term cultures and the identification of various phenotypes of stromal mesenchyme

The in vivo observations of Friedenstein obtained strong support from the studies of TM Dexter and colleagues in Manchester during the late 1970's. This research group discovered the means to obtain long-term hemopoiesis in vitro, mimicking a bone marrow like situation under culture conditions [15-19]. Hemopoiesis was studied in vitro for many years, and is still being studied extensively, using clonogenic assays in which the stem cells are diluted such that upon induction with a cytokine they proliferate to form an isolated colony. This allowed a powerful means to quantify processes of cell response to cytokines. However, this approach completely ignores the fact that within hemopoietic organs stem cells respond to cytokines while in intimate contact among themselves, and also with the organ stromata. Dexter et al, showed that seeding of dense bone marrow cultures, under specific conditions, including selected horse sera, relatively low temperature and supplementation of hydrocortisone, lead to the formation of an adherent layer of mesenchymal stromata and associated hemopoiesis, that lasted for many months. In these long-term cultures, large numbers of hemopoietic progenitor cells were produced. One important aspect of these long-term cultures was the heterogeneity of stromal cell types observed (Figure 2). Myelopoiesis was associated with "giant fat cells" and macrophages were observed underneath flat "blanket cells", to mention just two examples. Thus, along with the heterogeneity of hemopoietic cell types, a multitude of mesenchymal "types" was observed. Although the studies of long-term bone marrow cultures did not discover the means to segregate between mesenchymal cell types, they did highlight the possibility that such heterogeneity that may have functional significance, does exist.

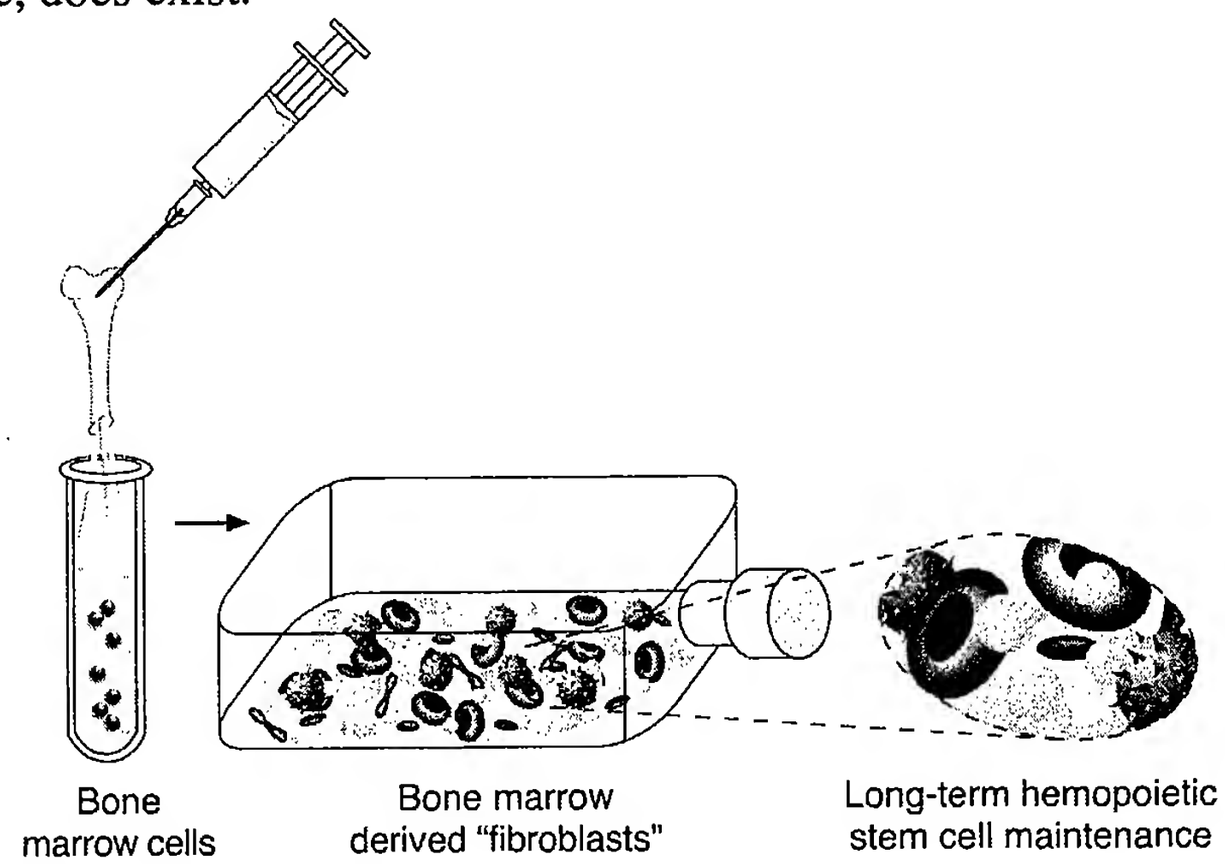

Figure 2: Dexter's long-term bone marrow cultures- Bone marrow cells cultured at high densities form an in vitro simulation of a hemopoietic tissue in which stem cells are maintained and differentiate for months of continuous incubation. This process is highly dependent on the development of an adherent layer of supportive stroma made of a variety of mesenchymal phenotypes.

Stromal cell lines and further analysis of mesenchymal phenotypes

During the early 1980's we were engaged in attempts to derive adherent cell lines from bone marrow cultures with the aim of cloning them, to arrive at a point of better characterization of mesenchymal phenotypes, or cell types. These attempts yielded a series of cell lines; the mouse bone marrow adherent (MBA) cell line series [20-22]. Analysis of clonal populations revealed great resemblance between the different clones, along with clear differences. According to morphology, intracellular enzymes and extracellular matrix components, the cells lines were designated as: endothelial like, adipogenic, fibroblastoid, fibro-endothelial, osteogenic and macrophages [23] (Figure 3).

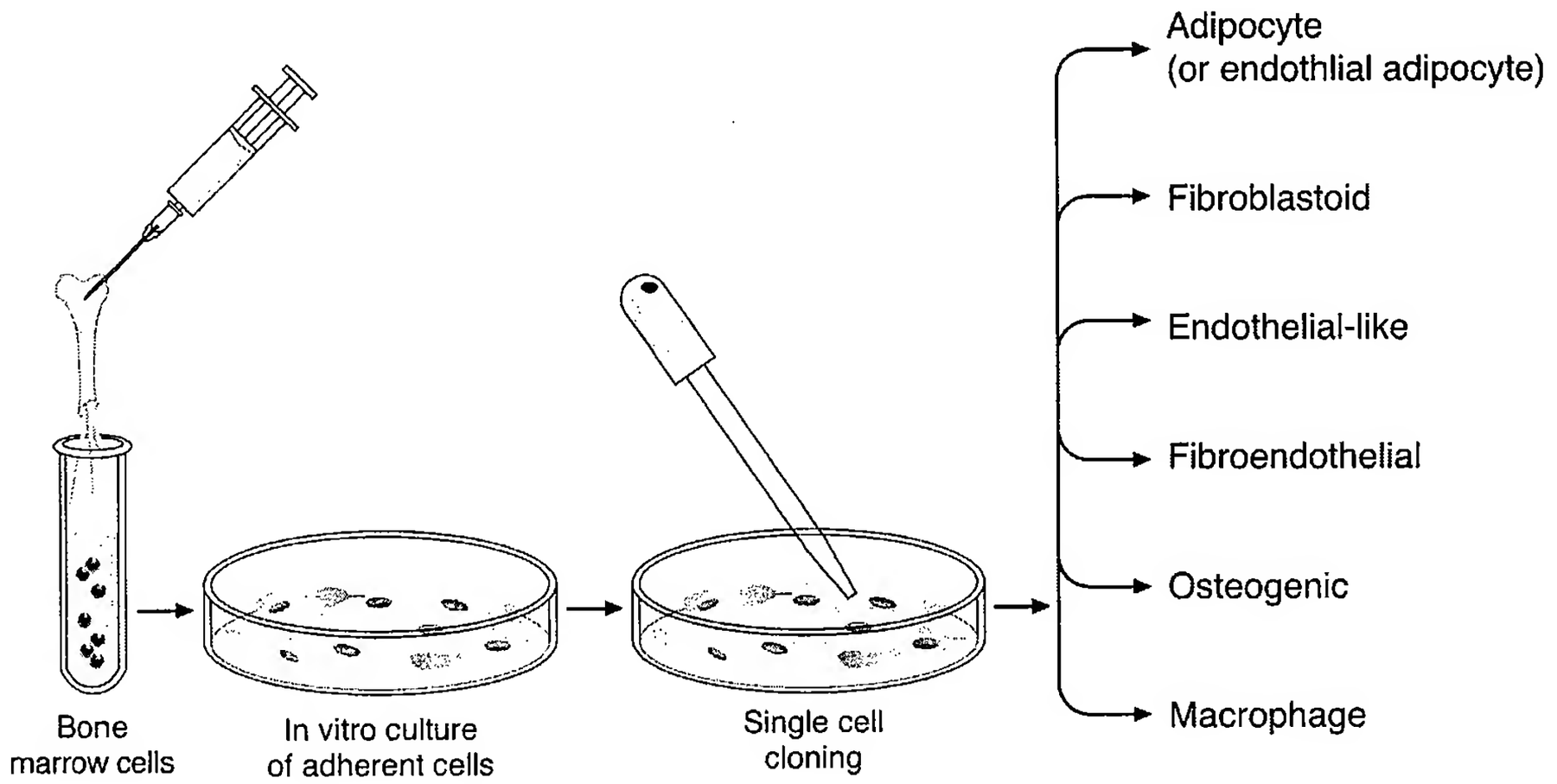

Figure 3: Adherent cell layers that result from in vitro seeding of bone marrow cells are a source for permanent cell line that can be propagated for years while maintaining a relatively fixed phenotype is maintained under constant and strict conditions. However, their phenotype can be easily shifted by changing the culture conditions.

With the exception of the latter that are from hemopoietic descent, all the rest are mesenchymal in nature although they clearly show properties of other lineages. The best example is the MBA-2.1 cell that is derived from bone marrow mesenchyme but shares properties with endothelium. Most of the cell lines shared osteogenic properties, but in some, such as the MBA-15, these were more pronounced compared to the others [24-26]. The capacity to support hemopoiesis was by far more restricted. Only the adipogenic cell lines exhibited this trait [27-29]. The derivation of cell lines with such diverse properties could suggest that the bone marrow mesenchyme is divided into different cell types. Indeed, it has been suggested that all these different phenotypes are derived from a single MSC. Yet, our study of these cell lines indicated that the cells tend to change phenotypes even when obtained as clonal populations. Enzyme expression varied extensively among individual cells of the same clone, adipogenesis was an inducible and reversible trait in all of the clones, and the capacity to support hemopoiesis could be induced or lost according to the in vitro conditions used to grow the cells. Thus, the bottom line

conclusion of the study of bone marrow derived mesenchymal cell lines is that their phenotype is very flexible [30]. Contrary to hemopoietic cell lines that upon in vitro derivation maintain a relatively fixed phenotype, with the exception of phenomena of lineage promiscuity, the study of cultured mesenchymal cell lines lead to the conclusion that these cells continue to change fate upon culture, and are thus highly plastic.

Accumulation of evidence for mesenchymal plasticity

The study of bone marrow derived mesenchymal cell lines thus confirmed that highly variable phenotypes of adherent cells that arise in bone marrow cultures. These experiments could be interpreted in two ways: the heterogeneity observed could result from the presence of many types of cells having different origins, or alternatively, these different phenotypes could be derived from a common stem cell. This latter conclusion gained support from cloning experiments. Pittenger et al. propagated single cells from human bone marrow that were expanded to form colonies. Cells from these clones could differentiate into adipocytes, chondrogenic and osteocytic cells, thus confirming the multipotency of the mesenchymal colony forming cell [31]. This rather limited differentiation potential was dramatically widened following the discovery of Jiang et al. that a cell population from the adult bone marrow, that was also found in the brain and muscle, termed multipotential adult progenitor cells (MAPC), co-purify with MSC and thus maybe a subtype of this population, and is characterized by unexpected pluripotency; by analysis at the single cell level and genetic labeling of the cells the investigators confirmed that MAPC differentiate in vitro into mesodermal derivatives including endothelium, neuroectoderm and endoderm cells. Moreover, upon inoculation into mouse blastocyst, these cells contributed to the formation of a multitude of somatic cells [32-35]. Such cells were characterized in the mouse, rat and man [36]. These studies were followed by a series of publications that support the pluripotent nature of mesenchymal cells. Marrow isolated adult multilineage inducible (MIAMI) cells reside in human bone marrow and upon in vitro isolation differentiate into osteoblasts, chondrocytes, adipocytes, neural cells and cells expressing genes associated with pancreatic islet phenotype [37]. In a separate study, small airway epithelial cells were heat shocked to cause injury. The cell damage was corrected by the addition of human MSCs that differentiated into epithelial-like cells as indicated by expression of epithelial genes. Along with such direct differentiation, cell fusion was observed and accounted for part of the contribution of the MSC to epithelial repair [38]. Like other phenomena of cell plasticity this phenomenon suggests a possible use of MSC in cell therapy, and in this case, lung diseases such as cystic fibrosis [39]. Additional reports focused on neuronal differentiation of bone marrow stromal cells. Such cells were shown to readily differentiate into neurons [40]. An independent study indicated that it is possible to derive neural cells from human bone marrow stoma with high efficiency; MSC were isolated, grown in neurosphere-like structures and were shown to differentiate into astroglia, oligodendroglia and neurons [41]. Transplantation of rat marrow stromal cells under xenogenic conditions, into chick embryos, resulted in expansion of the transplanted population and integration into the heart and other tissues. Some of the donor cells in the heart expressed heart muscle markers [42]. It has been shown that MSC are capable of migrating into the heart upon systemic administration [43]. Kawada et al. have isolated a

clonal population of cardiomyogenic cells from MSC. They have compared the capacity of isolated hemopoietic stem cells to contribute to ischemic myocardium following myocardial infarction. The study indicated that HSC do not contribute much, whereas the cardiomyogenic cell line generated actinin positive heart cells [44]. Whether this property is indeed shared by primary MSC remains to be examined. A recent publication by Yokoo et al. showed that human bone marrow derived MSCs participate in the kidney organogenesis upon injection into rodent embryos [45]. These cells seem, therefore, to undergo a transdifferentiation process to form entire nephrons. Thus, similar to the MET that occurs during normal development in kidney organogenesis, bone marrow mesenchyme from an adult source maintains its plastic properties that are exhibited upon introduction into a permissive embryonic environment (Figure 4) (for additional review of mesenchymal stem cells see [46-48]).

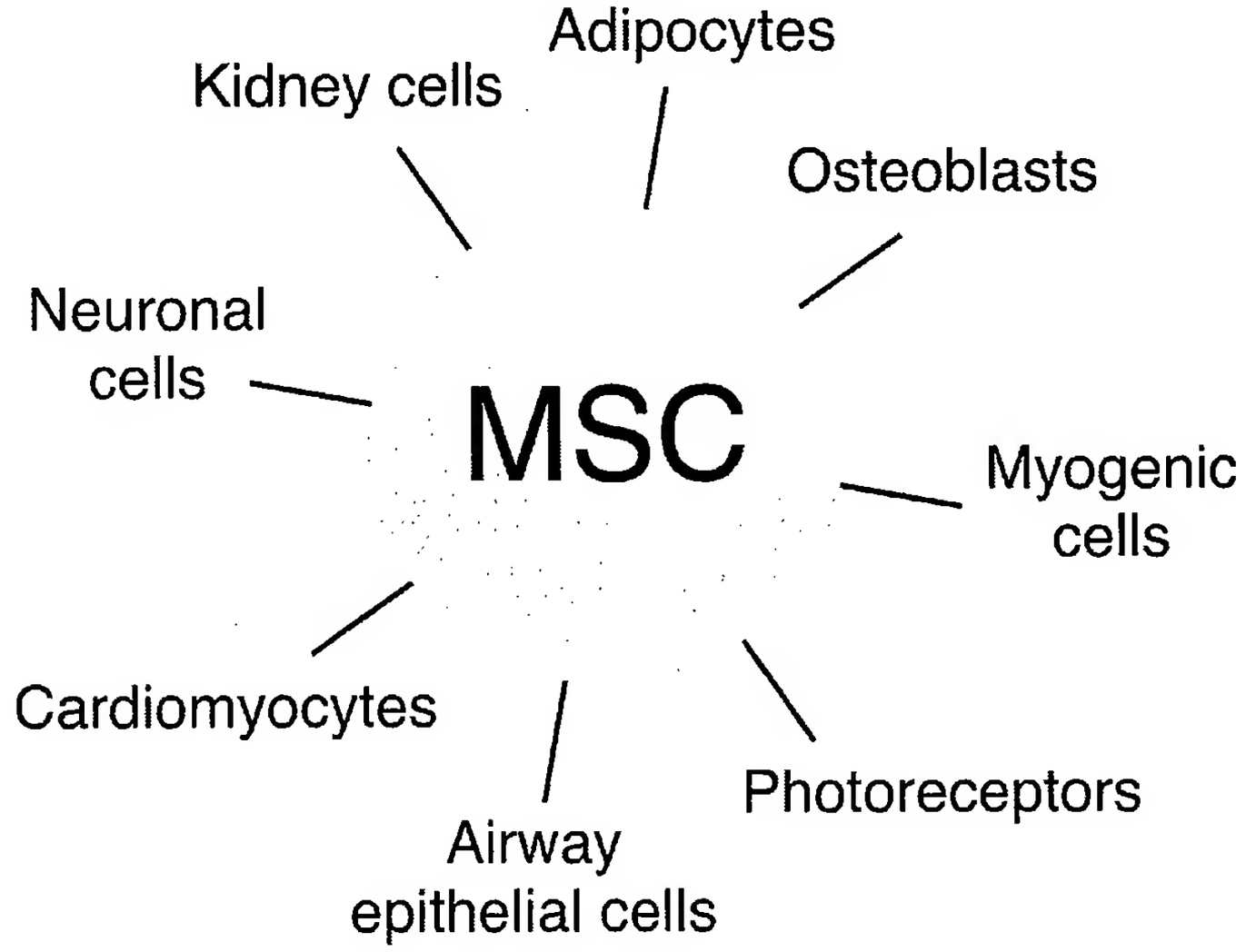

Figure 4: Bone marrow derived stromal mesenchyme from mouse, or MSC, rat and human origin has plastic nature as revealed by their capacity to differentiate into a multitude of cell types traditionally classified as belonging to the three embryonic germ layers. The figure depicts directions of differentiation reported by different laboratories, as discussed in the text.

Why are pluripotent mesenchymal stem cells so abundant in hemopoietic organs?

Mesenchymal stem cells have been isolated from the embryo as well as from the adult. In adult organism MSC have been usually isolated from hemopoietic organs, particularly from the bone marrow. However, such cells are also found in other organs and the type

called MAPC was also isolated from muscle and brain [33]. MSC are found also in the spleen and thymus and we have derived mesenchymal cell lines from thymic origin that were very similar in their properties to bone marrow derived stroma [49,50]. MSC were found in fatty tissues and are probably found in additional sites. However, they seem to be found in the bone marrow in a relatively higher proportion. The bone marrow space may be therefore used as a depository and a possible source for stem cells that could be recruited to other sites in the body, upon injury. Many studies in the mouse and in humans suggest that stromal mesenchyme may indeed be transplantable [51-57] although there is an ongoing controversy regarding this issue. Are those cells also migrating in a physiological manner? In fact, there are several indications that this is the case. First, the bone marrow was reported to be the source for endothelial precursors that gave rise to capillaries in solid tumors [58]. Furthermore, gastric ulcers induced by *Helicobactor pylori*, attract, by an unknown mechanism, bone marrow derived cells, probably MSC, that travel to the ulcer, transdifferentiate there to gastric epithelium and eventually transform into gastric adenocarcinomata [59]. The investigators proposed that tumor formation, in general, may be mediated by such migration of cells from the bone marrow. Several additional reports support the notion that mesenchymal cells are migratory and that this migration is mediated by the chemokine receptor CXCR4 [60,61]. Obviously, assisting tumor formation could not be the physiological role of bone marrow mesenchymal cells. Alternatively, they may be normally recruited to injury sites to mend damages. If the process is unsuccessful or not properly restrained, tumors may arise. In addition, I would like to propose that under milder conditions, when injury is restricted to the loss of a few cells within a tissue, correction of the damage may be done by resident MSC without the need to recruit cells from the bone marrow. The selection of the bone marrow as a depository site for stem cells, that occurred during evolution, is probably due to the fact that it is in the crossroad of the vascular system and constantly seeds the circulation and the peripheral organs with derivatives of the hemopoietic stem cell. The bone marrow structure is thus ideal for responding to demand, due to tissue damage, by fast release of cells capable of traveling through the vasculature and of contributing to the correction of damages. Therefore, the adult mesenchyme is not different in principle from embryonic mesenchyme and the existing differences are primarily quantitative (Figure 5). In the embryo tissue and organ constructions are dramatic and robust processes, they entail very extensive plastic cell behavior. By contrast, the adult organism requires relatively little changes, mostly related to tissue maintenance in replacement of damaged or aged cells. These processes occur at a low incidence and are much more modest, compared to those in the embryo. Therefore, the plastic nature of adult MSC is suppressed and revealed only upon their in vitro culture when tissue constrains are relived.

The stem state and mesenchymal plasticity

I recently suggested that stemness is a state that, theoretically, any cell may enter (Figure 6). Thus a stem cell, as a stable cellular entity, does not exist. Rather, stemness is an unstable state characterized by promiscuous gene expression that puts the cell in a standby state, ready to commit to a variety of different directions. Analysis of all the different traits ascribed to stem cells, i.e. the capacity to self-renew, or to self-renew

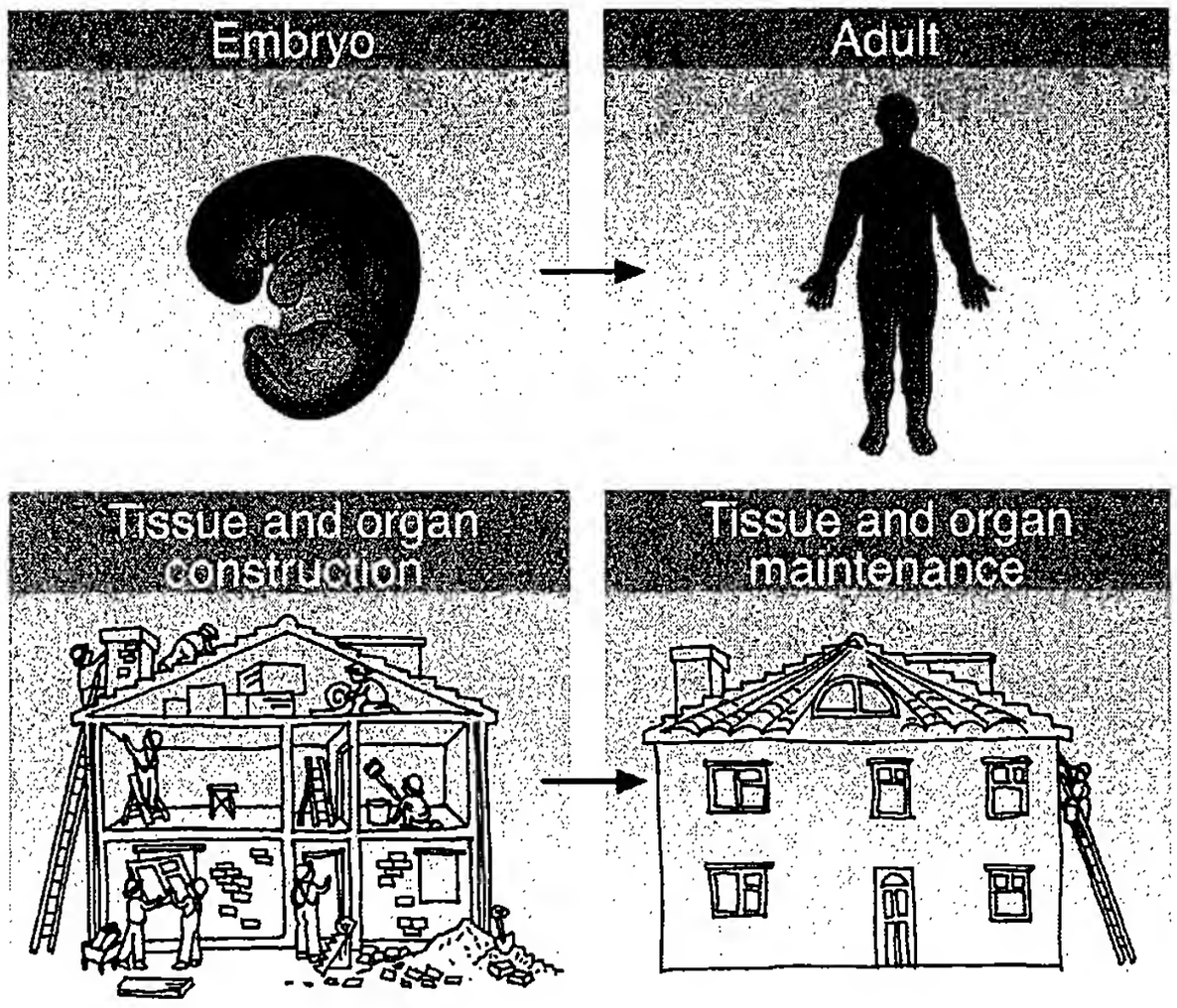

Figure 5: Mesenchymal functions in the embryo and the adult differ quantitatively only- Whereas in the embryo the mesenchyme is involved in robust processes of tissue and organ construction, the task of these cells in the adult may be to mend small tissue and organ damages. House illustrations in the lower panels: During constructions many builders are employed and use a series of tools and methods which are operated extensively and robustly to reach the final goal, a complete house in a limited time frame. This finished facility should however be further maintained throughout the years. Naturally, only few workers would be needed. The tools and methods used for house maintenance are identical or similar to those used for constructions yet they are employed on a small scale and with much caution, so as not to cause damage to the already functional home.

indefinitely, the capacity to proliferate extensively, etc, seem to be options rather than a true prerequisites of stemness. I recently suggested that the stem state entails pluripotency and plasticity, rather than the other properties commonly ascribed to stem cells [9,10]. The MSC demonstrates this notion very well: mesenchymal cells shift fats, and as discussed above, may differentiate into one cell type while maintaining the ability to go back into the original "stem cell" phenotype. Further, mesenchymal cells express a variety of genes that are supposed to characterize their differentiated progeny. Transcriptome analysis shows in fact that stem cells express the majority of their genome. Most importantly, mesenchymal cells are pluripotent and highly plastic, in that they may give rise to cells of all three embryonic germ layers.

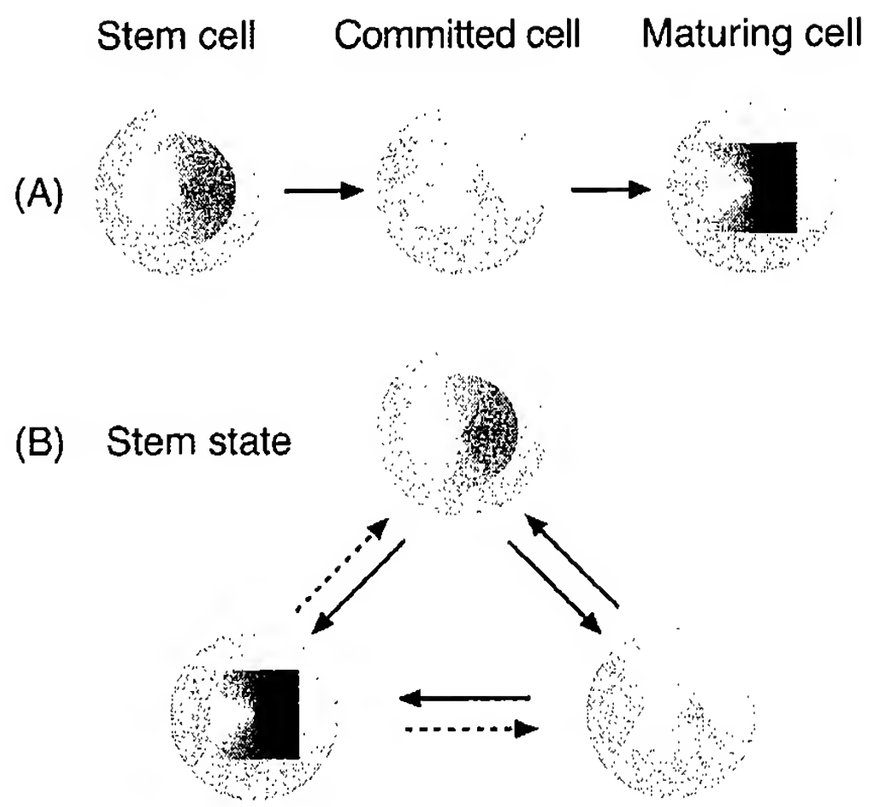

Figure 6: The traditional stem cell model, assuming the stem cell to be an origin of a hierarchy of descending potency for renewal (A) as opposed to the stem state notion in which cells may assume a stem state even when already in a differentiating stage (B).

Acknowledgements

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References

1. Prindull G, Zipori D (2004) Environmental guidance of normal and tumor cell plasticity: epithelial mesenchymal transitions as a paradigm. Blood 8:8
2. Taylor SM, Jones PA (1979) Multiple new phenotypes induced in 10T1/2 and 3T3 cells treated with 5-azacytidine. Cell 17:771
3. Wang EA, Israel DI, Kelly S, Luxenberg DP (1993) Bone morphogenetic protein-2 causes commitment and differentiation in C3H10T1/2 and 3T3 cells. Growth Factors 9:57
4. Denker AE, Nicoll SB, Tuan RS (1995) Formation of cartilage-like spheroids by micromass cultures of murine C3H10T1/2 cells upon treatment with transforming growth factor-beta 1. Differentiation 59:25

5. O'Donoghue K, Choolani M, Chan J, de la Fuente J, Kumar S, Campagnoli C, Bennett PR, Roberts IA, Fisk NM (2003) Identification of fetal mesenchymal stem cells in maternal blood: implications for non-invasive prenatal diagnosis. Mol Hum Reprod 9:497
6. Khosrotehrani K, Bianchi DW (2005) Multi-lineage potential of fetal cells in maternal tissue: a legacy in reverse. J Cell Sci 118:1559
7. Mendes SC, Robin C, Dzierzak E (2005) Mesenchymal progenitor cells localize within hematopoietic sites throughout ontogeny. Development 132:1127
8. Kues WA, Petersen B, Mysegades W, Carnwath JW, Niemann H (2004) Isolation of Murine and Porcine Fetal Stem Cells from Somatic Tissue. Biol Reprod
9. Zipori D (2004) The nature of stem cells: state rather than entity. Nat Rev Genet 5:873
10. Zipori D (2005) The stem state: plasticity is essential, while self-renewal and hierarchy are optional. Stem Cells
11. Friedenstein AJ, Chailakhjan RK, Lalykina KS (1970) The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 3:393
12. Friedenstein AJ, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luria EA, Ruadkow IA (1974) Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. Exp Hematol 2:83
13. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV (1974) Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. Transplantation 17:331
14. Tavassoli M, Friedenstein A (1983) Hemopoietic stromal microenvironment. Am J Hematol 15:195
15. Dexter TM, Wright EG, Krizsa F, Lajtha LG (1977) Regulation of haemopoietic stem cell proliferation in long term bone marrow cultures. Biomedicine 27:344
16. Allen TD, Dexter TM (1982) Ultrastructural aspects of erythropoietic differentiation in long-term bone marrow culture. Differentiation 21:86
17. Allen TD, Dexter TM (1983) Long term bone marrow cultures: an ultrastructural review. Scan Electron Microsc:1851
18. Dexter TM, Coutinho LH, Spooncer E, Heyworth CM, Daniel CP, Schiro R, Chang J, Allen TD (1990) Stromal cells in haemopoiesis. Ciba Found Symp 148:76
19. Testa NG, Dexter TM (1991) The biology of long-term bone marrow cultures and its application to bone marrow transplantation. Curr Opin Oncol 3:272
20. Zipori D, Friedman A, Tamir M, Silverberg D, Malik Z (1984) Cultured mouse marrow cell lines: interactions between fibroblastoid cells and monocytes. J Cell Physiol 118:143
21. Zipori D, Duksin D, Tamir M, Argaman A, Toledo J, Malik Z (1985) Cultured mouse marrow stromal cell lines. II. Distinct subtypes differing in morphology, collagen types, myelopoietic factors, and leukemic cell growth modulating activities. J Cell Physiol 122:81
22. Zipori D, Toledo J, von der Mark K (1985) Phenotypic heterogeneity among stromal cell lines from mouse bone marrow disclosed in their extracellular matrix composition and interactions with normal and leukemic cells. Blood 66:447

23. Zipori D (1989) Cultured stromal cell lines from hemopoietic tissues. In: T M (ed) Blood Cell Formation: The Role of the Hemopoietic Microenvironment. Clifton, NY: Humana Press, 287
24. Knospe WH, Husseini SG, Zipori D, Fried W (1993) Hematopoiesis on cellulose ester membranes. XIII. A combination of cloned stromal cells is needed to establish a hematopoietic microenvironment supportive of trilineal hematopoiesis. Exp Hematol 21:257
25. Benayahu D, Fried A, Zipori D, Wientroub S (1991) Subpopulations of marrow stromal cells share a variety of osteoblastic markers. Calcif Tissue Int 49:202
26. Benayahu D, Gurevitch O, Zipori D, Wientroub S (1994) Bone formation by marrow osteogenic cells (MBA-15) is not accompanied by osteoclastogenesis and generation of hematopoietic supportive microenvironment. J Bone Miner Res 9:1107
27. Zipori D, Lee F (1988) Introduction of interleukin-3 gene into stromal cells from the bone marrow alters hemopoietic differentiation but does not modify stem cell renewal. Blood 71:586
28. Tamir M, Harris N, Trainin N, Toledo J, Zipori D (1989) Multilineage hemopoiesis induced by cloned stromal cells. Int J Cell Cloning 7:373
29. Umiel T, Friedman S, Zaizov R, Cohen IJ, Gozes Y, Epstein N, Kobiler D, Zipori D (1986) Long-term culture of infant leukemia cells: dependence upon stromal cells from the bone marrow and bilineage differentiation. Leuk Res 10:1007
30. Sternberg D, Peled A, Shezen E, Abramsky O, Jiang W, Bertolero F, Zipori D (1996) Control of stroma-dependent hematopoiesis by basic fibroblast growth factor: stromal phenotypic plasticity and modified myelopoietic functions. Cytokines Mol Ther 2:29
31. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143
32. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418:41
33. Jiang Y, Vaessen B, Lenvik T, Blackstad M, Reyes M, Verfaillie CM (2002) Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. Exp Hematol 30:896
34. Keene CD, Ortiz-Gonzalez XR, Jiang Y, Largaespada DA, Verfaillie CM, Low WC (2003) Neural differentiation and incorporation of bone marrow-derived multipotent adult progenitor cells after single cell transplantation into blastocyst stage mouse embryos. Cell Transplant 12:201
35. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM (2002) Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest 109:337
36. Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu WS, Verfaillie CM (2002) Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest 109:1291
37. D'Ippolito G, Diabira S, Howard GA, Menei P, Roos BA, Schiller PC (2004) Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of

postnatal young and old human cells with extensive expansion and differentiation potential. J Cell Sci 117:2971
38. Spees JL, Olson SD, Ylostalo J, Lynch PJ, Smith J, Perry A, Peister A, Wang MY, Prockop DJ (2003) Differentiation, cell fusion, and nuclear fusion during ex vivo repair of epithelium by human adult stem cells from bone marrow stroma. Proc Natl Acad Sci U S A 100:2397
39. Wang G, Bunnell BA, Painter RG, Quiniones BC, Tom S, Lanson NA, Jr., Spees JL, Bertucci D, Peister A, Weiss DJ, Valentine VG, Prockop DJ, Kolls JK (2005) Adult stem cells from bone marrow stroma differentiate into airway epithelial cells: Potential therapy for cystic fibrosis. Proc Natl Acad Sci U S A 102:186
40. Levy YS, Merims D, Panet H, Barhum Y, Melamed E, Offen D (2003) Induction of neuron-specific enolase promoter and neuronal markers in differentiated mouse bone marrow stromal cells. J Mol Neurosci 21:121
41. Hermann A, Gastl R, Liebau S, Popa MO, Fiedler J, Boehm BO, Maisel M, Lerche H, Schwarz J, Brenner R, Storch A (2004) Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells. J Cell Sci 117:4411
42. Pochampally RR, Neville BT, Schwarz EJ, Li MM, Prockop DJ (2004) Rat adult stem cells (marrow stromal cells) engraft and differentiate in chick embryos without evidence of cell fusion. Proc Natl Acad Sci U S A 101:9282
43. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J (2003) Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. Circulation 108:863
44. Kawada H, Fujita J, Kinjo K, Matsuzaki Y, Tsuma M, Miyatake H, Muguruma Y, Tsuboi K, Itabashi Y, Ikeda Y, Ogawa S, Okano H, Hotta T, Ando K, Fukuda K (2004) Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood 104:3581
45. Yokoo T, Ohashi T, Shen JS, Sakurai K, Miyazaki Y, Utsunomiya Y, Takahashi M, Terada Y, Eto Y, Kawamura T, Osumi N, Hosoya T (2005) Human mesenchymal stem cells in rodent whole-embryo culture are reprogrammed to contribute to kidney tissues. Proc Natl Acad Sci U S A 102:3296
46. Zipori D (2004) Mesenchymal stem cells: harnessing cell plasticity to tissue and organ repair. Blood Cells Mol Dis 33:211
47. Gerson SL (1999) Mesenchymal stem cells: no longer second class marrow citizens. Nat Med 5:262
48. Deans RJ, Moseley AB (2000) Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 28:875
49. Tamir M, Rozenszajn LA, Malik Z, Zipori D (1987) Thymus-derived stromal cell lines. Int J Cell Cloning 5:289
50. Tamir M, Eren R, Globerson A, Kedar E, Epstein E, Trainin N, Zipori D (1990) Selective accumulation of lymphocyte precursor cells mediated by stromal cells of hemopoietic origin. Exp Hematol 18:322
51. Simmons PJ, Przepiorka D, Thomas ED, Torok-Storb B (1987) Host origin of marrow stromal cells following allogeneic bone marrow transplantation. Nature 328:429

52. Laver J, Jhanwar SC, O'Reilly RJ, Castro-Malaspina H (1987) Host origin of the human hematopoietic microenvironment following allogeneic bone marrow transplantation. Blood 70:1966
53. Pereira RF, O'Hara MD, Laptev AV, Halford KW, Pollard MD, Class R, Simon D, Livezey K, Prockop DJ (1998) Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. Proc Natl Acad Sci U S A 95:1142
54. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK (1999) Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 5:309
55. Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R (2003) Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. Blood 101:2999
56. Anjos-Afonso F, Siapati EK, Bonnet D (2004) In vivo contribution of murine mesenchymal stem cells into multiple cell-types under minimal damage conditions. J Cell Sci 117:5655
57. Allers C, Sierralta WD, Neubauer S, Rivera F, Minguell JJ, Conget PA (2004) Dynamic of distribution of human bone marrow-derived mesenchymal stem cells after transplantation into adult unconditioned mice. Transplantation 78:503
58. Peters BA, Diaz LA, Polyak K, Meszler L, Romans K, Guinan EC, Antin JH, Myerson D, Hamilton SR, Vogelstein B, Kinzler KW, Lengauer C (2005) Contribution of bone marrow-derived endothelial cells to human tumor vasculature. Nat Med 11:261
59. Houghton J, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR, Wang TC (2004) Gastric cancer originating from bone marrow-derived cells. Science 306:1568
60. Ji JF, He BP, Dheen ST, Tay SS (2004) Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. Stem Cells 22:415
61. Wynn RF, Hart CA, Corradi-Perini C, O'Neill L, Evans CA, Wraith JE, Fairbairn LJ, Bellantuono I (2004) A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. Blood 104:2643

APPENDIX 3

NCBI Nucleotide banner

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Range: from begin to end Reverse complemented strand Features: + Refresh

This record may be shown in a reduced form. Use 'Hide' checkboxes for control.

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                     /db_xref="taxon:9606"
                     /clone="CBG1"
                     /cell_line="CGM1, either haplotype A or B"
                     /clone_lib="Kai Wang's"
                     /germline
                     /note="(vector PWE15A)"
     source          195003..198074
                     /organism="Homo sapiens"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9606"
                     /clone="CBG1/C29gap"
                     /germline
                     /note="The gap between cosmid clones CBG1 and C29 was
                     closed by the sequencing of a PCR reaction product."
     source          197962..232650
                     /organism="Homo sapiens"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9606"
                     /clone="C29"
                     /cell_line="ATCC1475"
                     /clone_lib="Kai Wang's"
                     /germline
                     /note="(vector PWE15A)"
     misc_feature    1..232650
                     /note="This sequence overlaps section 2 of the human T
                     cell receptor beta locus, Genbank Accession Number U66060,
                     by 20616 bases."
     repeat_region   957..1082
                     /note="LINE; putative"
                     /rpt_family="L1MA4A"
     repeat_region   1294..1328
                     /note="SINE; putative"
                     /rpt_family="MIR"
     gene            1483..1997
                     /gene="TCRBV17S1A1T"
                     /note="proposed new name: TCRBV19S1"
     CDS             join(1483..1531,1664..>1958)
                     /gene="TCRBV17S1A1T"
                     /codon_start=1
                     /product="V_segment translation product"
                     /protein_id="AAC80216.1"
                     /db_xref="GI:2218050"
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V_segment       join(1483..1531,1664..1958)
                /gene="TCRBV17S1A1T"
                /standard_name="TCRBV17S1"
misc_recomb     1959..1965
                /gene="TCRBV17S1A1T"
                /note="RSS_heptamer"
misc_recomb     1966..1988
                /gene="TCRBV17S1A1T"
                /note="RSS_spacer"
misc_recomb     1989..1997
                /gene="TCRBV17S1A1T"
                /note="RSS_nonamer"
repeat_region   complement(2280..2579)
                /note="putative"
                /rpt_family="Alu"
repeat_region   2664..2756
                /note="(TA)46, 81% conserved; putative"
                /rpt_type=tandem
repeat_region   4775..5156
                /note="LINE; putative"
                /rpt_family="L1ME3"
repeat_region   complement(5186..5635)
                /note="MaLR retroposon LTR; putative"
                /rpt_family="MLT1F/G"
misc_feature    5584..>5584
                /note="translocation breakpoint; t(7:9); putative.  Begin
                region of similarity to chromosome 9p13."
misc_feature    6654..6746
                /standard_name="TCRBV relic (exon 2 only)"
                /note="
                78% similar to TCRBV7 (4) variable gene exon2 regions;
                putative"
repeat_region   7426..7605
                /note="DNA transposon fossil; putative"
                /rpt_family="MER47"
repeat_region   complement(8681..8975)
                /note="putative"
                /rpt_family="Alu"
gene            9153..9864
                /gene="TCRBV2S1"
                /note="proposed new name: TCRBV20S1"
CDS             join(9153..9183,9522..>9825)
                /gene="TCRBV2S1"
                /codon_start=1
                /product="V_segment translation product"
                /protein_id="AAC80217.1"
                /db_xref="GI:2218051"
V_segment       join(9153..9183,9522..9825)
                /gene="TCRBV2S1"
                /standard_name="TCRBV2S1"
misc_recomb     9826..9832
                /gene="TCRBV2S1"
                /note="RSS_heptamer"
misc_recomb     9833..9855
                /gene="TCRBV2S1"
                /note="RSS_spacer"
misc_recomb     9856..9864
                /gene="TCRBV2S1"
                /note="RSS_nonamer"
repeat_region   10771..11307
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                     /note="LINE; putative"
                     /rpt_family="L1ME"
repeat_region        11431..11645
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER63"
repeat_region        12880..12923
                     /note="putative"
                     /rpt_unit_seq="ac"
repeat_region        complement(13511..13698)
                     /note="LINE; putative"
                     /rpt_family="MIR2"
repeat_region        13836..14352
                     /note="Unclassified; putative"
                     /rpt_family="MER34"
repeat_region        15731..16019
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        16056..16351
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        16432..17709
                     /note="LINE; putative"
                     /rpt_family="L1ME"
repeat_region        17987..18281
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        18267..18303
                     /note="putative"
                     /rpt_unit_seq="aaat"
repeat_region        18423..19033
                     /note="putative"
                     /rpt_family="LINE 1"
repeat_region        18443..19041
                     /note="LINE; putative"
                     /rpt_family="L1PA7"
gene                 19235..19835
                     /gene="TCRBV10S1P"
                     /note="proposed new name: TCRBV21S1.  This gene has a
                     frameshift in exon 1."
                     /pseudo
CDS                  join(19336..19385,19499..>19796)
                     /gene="TCRBV10S1P"
                     /pseudo
                     /codon_start=1
V_segment            join(19336..19385,19499..19796)
                     /gene="TCRBV10S1P"
                     /standard_name="TCRBV10S1"
                     /pseudo
misc_recomb          19797..19803
                     /gene="TCRBV10S1P"
                     /note="RSS_heptamer"
misc_recomb          19804..19826
                     /gene="TCRBV10S1P"
                     /note="RSS_spacer"
misc_recomb          19827..19835
                     /gene="TCRBV10S1P"
                     /note="RSS_nonamer"
repeat_region        complement(20366..20691)
                     /note="putative"
                     /rpt_family="Alu"
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repeat_region   complement(20775..20909)
                /note="LINE; putative"
                /rpt_family="L1ME2"
misc_feature    complement(20988..21444)
                /note="putative processed EB1 pseudogene fragment; 78%
                similar to 3' end of the humanEB1 mRNA (U24166); putative"
repeat_region   21457..21578
                /note="LINE; putative"
                /rpt_family="L1MC1"
repeat_region   complement(23424..23654)
                /note="putative"
                /rpt_family="MIR"
repeat_region   23965..24011
                /note="SINE; putative"
                /rpt_family="MIR"
gene            24076..24646
                /gene="TCRBV29S1P"
                /note="Proposed new name: TCRBV22S1.  This gene has a
                frameshift in exon 2."
                /pseudo
CDS             join(24157..24205,24338..>24407)
                /gene="TCRBV29S1P"
                /pseudo
                /codon_start=1
V_segment       join(24157..24205,24338..24407)
                /gene="TCRBV29S1P"
                /standard_name="TCRBV29S1"
                /pseudo
misc_recomb     24608..24614
                /gene="TCRBV29S1P"
                /note="RSS_heptamer"
misc_recomb     24615..24637
                /gene="TCRBV29S1P"
                /note="RSS_spacer"
misc_recomb     24638..24646
                /gene="TCRBV29S1P"
                /note="RSS_nonamer"
repeat_region   complement(24962..25003)
                /note="LINE; putative"
                /rpt_family="MIR2"
repeat_region   26023..27210
                /note="LINE; putative"
                /rpt_family="L1ME"
gene            28303..28911
                /gene="TCRBV19S1P"
                /note="proposed new name: TCRBV23S1.  This gene has a
                defective splice donor."
                /pseudo
CDS             join(28376..28424,28575..>28872)
                /gene="TCRBV19S1P"
                /pseudo
                /codon_start=1
V_segment       join(28376..28424,28575..28872)
                /gene="TCRBV19S1P"
                /standard_name="TCRBV19S1"
                /pseudo
repeat_region   28486..28522
                /note="(TTTGT)7; putative"
                /rpt_type=tandem
misc_recomb     28873..28879
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                     /gene="TCRBV19S1P"
                     /note="RSS_heptamer"
misc_recomb          28880..28902
                     /gene="TCRBV19S1P"
                     /note="RSS_spacer"
misc_recomb          28903..28911
                     /gene="TCRBV19S1P"
                     /note="RSS_nonamer"
repeat_region        complement(30000..30172)
                     /note="LINE; putative"
                     /rpt_family="L1ME"
repeat_region        complement(31310..32278)
                     /note="LINE; putative"
                     /rpt_family="L1M"
repeat_region        32293..35179
                     /note="LINE; putative"
                     /rpt_family="L1PA10"
variation            32295
                     /note="cosmid C215: a; cosmid G1: c"
                     /replace="c"
repeat_region        32756..35177
                     /note="putative"
                     /rpt_family="LINE 1"
repeat_region        complement(35452..35501)
                     /note="LINE; putative"
                     /rpt_family="L1"
repeat_region        36133..36852
                     /note="putative"
                     /rpt_family="HUMERSP2A"
gene                 37616..38232
                     /gene="TCRBV15S1"
                     /note="proposed new name: TCRBV24S1"
misc_feature         37616..37630
                     /gene="TCRBV15S1"
                     /note="conserved 16mer; possible promoter"
CDS                  join(37717..37765,37898..>38193)
                     /gene="TCRBV15S1"
                     /codon_start=1
                     /product="V_segment translation product"
                     /protein_id="AAC80218.1"
                     /db_xref="GI:2218052"
V_segment            join(37717..37765,37898..38193)
                     /gene="TCRBV15S1"
                     /standard_name="TCRBV15S1"
misc_recomb          38194..38200
                     /gene="TCRBV15S1"
                     /note="RSS_heptamer"
misc_recomb          38201..38223
                     /gene="TCRBV15S1"
                     /note="RSS_spacer"
misc_recomb          38224..38232
                     /gene="TCRBV15S1"
                     /note="RSS_nonamer"
repeat_region        39494..39681
                     /note="LINE; putative"
                     /rpt_family="MIR2"
repeat_region        39740..40011
                     /note="Unclassified; putative"
                     /rpt_family="MER71"
repeat_region        complement(40494..40880)
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                     /note="LINE; putative"
                     /rpt_family="L1PA16; fragment 1"
repeat_region        complement(40799..41461)
                     /note="putative"
                     /rpt_family="LINE 1"
repeat_region        complement(40881..41206)
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        complement(41207..41487)
                     /note="LINE; putative"
                     /rpt_family="L1PA16; fragment 2"
repeat_region        complement(41663..41709)
                     /note="LINE; putative"
                     /rpt_family="L1PA15; fragment 1"
repeat_region        complement(41739..42969)
                     /note="LINE; putative"
                     /rpt_family="L1PA15; fragment 2"
repeat_region        complement(41750..42978)
                     /note="putative"
                     /rpt_family="LINE 1"
repeat_region        42970..43711
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MARINER; fragment 1"
repeat_region        43712..44662
                     /note="endogenous retroviral LTR; putative"
                     /rpt_family="LTR13"
repeat_region        44663..45197
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MARINER; fragment 2"
repeat_region        complement(45198..45706)
                     /note="LINE; putative"
                     /rpt_family="L1PA15; fragment 2"
repeat_region        complement(45201..45695)
                     /note="putative"
                     /rpt_family="LINE 1"
repeat_region        45751..49033
                     /note="Mitochondrial insertion; D-loop region; putative"
                     /rpt_family="mitochondrial insertion sequence"
repeat_region        49535..49837
                     /note="putative"
                     /rpt_family="MER2"
repeat_region        complement(51517..51615)
                     /note="SINE; putative"
                     /rpt_family="MIR"
gene                 52003..52598
                     /gene="TCRBV11S1A1T"
                     /note="proposed new name: TCRBV25S1"
CDS                  join(52092..52140,52265..>52559)
                     /gene="TCRBV11S1A1T"
                     /codon_start=1
                     /product="V_segment translation product"
                     /protein_id="AAC80219.1"
                     /db_xref="GI:2218053"
V_segment            join(52092..52140,52265..52559)
                     /gene="TCRBV11S1A1T"
                     /standard_name="TCRBV11S1"
misc_recomb          52560..52566
                     /gene="TCRBV11S1A1T"
                     /note="RSS_heptamer"
misc_recomb          52567..52589
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                     /gene="TCRBV11S1A1T"
                     /note="RSS_spacer"
misc_recomb          52590..52598
                     /gene="TCRBV11S1A1T"
                     /note="RSS_nonamer"
repeat_region        53762..53869
                     /note="LINE; putative"
                     /rpt_family="MIR2"
repeat_region        54032..54363
                     /note="putative"
                     /rpt_family="MER2"
repeat_region        54702..55536
                     /note="putative"
                     /rpt_family="LINE 1"
repeat_region        54708..55541
                     /note="LINE; putative"
                     /rpt_family="L1PA5"
repeat_region        complement(55542..56168)
                     /note="LINE; putative"
                     /rpt_family="L1MB5-8; fragment 1"
repeat_region        complement(55677..57730)
                     /note="putative"
                     /rpt_family="LINE 1"
variation            55876
                     /note="cosmid G1: a; cosmid C68: g"
                     /replace="g"
repeat_region        56169..56471
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        complement(56473..57958)
                     /note="LINE; putative"
                     /rpt_family="L1MB5-8; fragment 2"
repeat_region        complement(57959..58163)
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER58B"
repeat_region        complement(58164..58536)
                     /note="LINE; putative"
                     /rpt_family="L1MB5-8; fragment 3"
variation            58519..58520
                     /note="cosmid G1: tt; cosmid C68: t"
                     /replace="t"
repeat_region        complement(58537..58832)
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        complement(58833..59422)
                     /note="LINE; putative"
                     /rpt_family="L1MB5-8; fragment 4"
repeat_region        59536..60105
                     /note="LINE; putative"
                     /rpt_family="L1M"
repeat_region        complement(60265..60423)
                     /note="LINE; putative"
                     /rpt_family="L1M"
repeat_region        60424..61036
                     /note="LINE; putative"
                     /rpt_family="L1MA8; fragment 1"
repeat_region        60592..62416
                     /note="putative"
                     /rpt_family="LINE 1"
repeat_region        61037..61577
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                     /note="putative"
                     /rpt_family="MLT2A"
variation            61565
                     /note="cosmid G1: a; cosmid C68: c"
                     /replace="c"
repeat_region        61578..62415
                     /note="LINE; putative"
                     /rpt_family="L1MA8; fragment 2"
gene                 62721..63213
                     /gene="TCRBV relic"
                     /note="This gene was formerly called TCRBV33S1."
V_segment            join(62721..62767,62806..63174)
                     /gene="TCRBV relic"
                     /pseudo
misc_recomb          63175..63181
                     /gene="TCRBV relic"
                     /note="RSS_heptamer"
misc_recomb          63182..63204
                     /gene="TCRBV relic"
                     /note="RSS_spacer"
misc_recomb          63205..63213
                     /gene="TCRBV relic"
                     /note="RSS_nonamer"
misc_feature         63572..63666
                     /note="STS; location of STS sWSS602 (G00251); putative"
repeat_region        64414..64575
                     /note="LINE; putative"
                     /rpt_family="MIR2"
repeat_region        64680..64846
                     /note="MaLR retroposon LTR; putative"
                     /rpt_family="MLT1D; fragment 1"
repeat_region        65096..65183
                     /note="LINE; putative"
                     /rpt_family="MIR2"
repeat_region        65185..65443
                     /note="MaLR retroposon LTR; putative"
                     /rpt_family="MLT1D; fragment 2"
repeat_region        65451..65959
                     /note="LINE; BLASTX similarity with LINE1 reverse
                     transcriptase; putative"
                     /rpt_family="LINE1-like sequence; fragment 1"
repeat_region        65960..66275
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        66276..66455
                     /note="LINE; BLASTX similarity with LINE1 reverse
                     transcriptase; putative"
                     /rpt_family="LINE1-like sequence; fragment 2"
repeat_region        66906..67016
                     /note="LINE; putative"
                     /rpt_family="MIR2"
repeat_region        67843..68566
                     /note="retroposon LTR; putative"
                     /rpt_family="MER72"
repeat_region        complement(68559..69111)
                     /note="putative"
                     /rpt_family="LINE 1"
repeat_region        complement(68567..69117)
                     /note="LINE; putative"
                     /rpt_family="L1"
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repeat_region   69627..69773
                /note="SINE; putative"
                /rpt_family="MIR"
repeat_region   complement(69838..70057)
                /note="SINE; putative"
                /rpt_family="MIR"
repeat_region   complement(70074..70466)
                /note="endogenous retroviral LTR; putative"
                /rpt_family="LTR14; fragment 1"
repeat_region   complement(70467..71503)
                /note="endogenous retrovirus, internal sequence; putative"
                /rpt_family="LTR14; fragment 2"
repeat_region   71505..71818
                /note="putative"
                /rpt_family="Alu"
repeat_region   complement(71822..72434)
                /note="endogenous retrovirus, internal sequence; putative"
                /rpt_family="LTR14; fragment 3"
repeat_region   72435..72685
                /note="putative"
                /rpt_family="Alu"
repeat_region   complement(72687..76461)
                /note="endogenous retrovirus, internal sequence; putative"
                /rpt_family="LTR14; fragment 4"
repeat_region   complement(76462..76852)
                /note="endogenous retroviral LTR; putative"
                /rpt_family="LTR14; fragment 5"
gene            77013..77536
                /gene="TCRBV28S1P"
                /note="proposed new name: TCRBV26S1.  This gene is missing
                a conserved cysteine in exon 2."
                /pseudo
CDS             join(77013..77061,77203..>77497)
                /gene="TCRBV28S1P"
                /pseudo
                /codon_start=1
V_segment       join(77013..77061,77203..77497)
                /gene="TCRBV28S1P"
                /standard_name="TCRBV28S1"
                /pseudo
misc_recomb     77498..77504
                /gene="TCRBV28S1P"
                /note="RSS_heptamer"
misc_recomb     77505..77527
                /gene="TCRBV28S1P"
                /note="RSS_spacer"
misc_recomb     77528..77536
                /gene="TCRBV28S1P"
                /note="RSS_nonamer"
repeat_region   77593..78441
                /note="putative"
                /rpt_family="MER4"
repeat_region   complement(78596..78935)
                /note="putative"
                /rpt_family="MLT1A"
repeat_region   complement(79417..79967)
                /note="MaLR retroposon LTR; putative"
                /rpt_family="MLT1E"
misc_difference 79662
                /note="cosmid C68 sequence should be cc, not c"
```

```
                     /replace="cc"
repeat_region        81672..82047
                     /note="putative"
                     /rpt_family="THE transposon like element"
repeat_region        complement(82071..82520)
                     /note="Unclassified; putative"
                     /rpt_family="MER74; fragment 1"
repeat_region        82521..83102
                     /note="Unclassified; putative"
                     /rpt_family="MER73"
repeat_region        83103..83405
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        complement(83428..83557)
                     /note="Unclassified; putative"
                     /rpt_family="MER74; fragment 2"
misc_feature         83938..>84379
                     /note="putative processed pseudogene fragment; similarity
                     to cDNA probably continues in either direction, but cDNA
                     sequence is not yet known; 95% similar to cDNAs R14537,
                     R18426, H24240; putative"
repeat_region        84300..84405
                     /note="LINE; putative"
                     /rpt_family="MIR2"
repeat_region        84520..84980
                     /note="putative"
                     /rpt_family="MLT1D"
repeat_region        complement(85107..85650)
                     /note="retroposon LTR; putative"
                     /rpt_family="MER41A; fragment 1"
repeat_region        complement(85651..86184)
                     /note="retroposon internal sequence; putative"
                     /rpt_family="MER41A; fragment 2"
repeat_region        complement(86351..88236)
                     /note="retroposon internal sequence; putative"
                     /rpt_family="MER41A; fragment 3"
repeat_region        87360..87397
                     /note="putative"
                     /rpt_type=tandem
                     /rpt_unit_seq="ta"
repeat_region        complement(88404..89752)
                     /note="retroposon internal sequence; putative"
                     /rpt_family="MER41A; fragment 4"
repeat_region        88664..88687
                     /note="(ATT)8; putative"
                     /rpt_type=tandem
repeat_region        complement(89753..90297)
                     /note="retroposon LTR; putative"
                     /rpt_family="MER41A; fragment 5"
repeat_region        complement(91044..91751)
                     /note="endogenous retroviral LTR; putative"
                     /rpt_family="MER50"
repeat_region        91953..92142
                     /note="SINE; putative"
                     /rpt_family="MIR"
repeat_region        complement(92197..92285)
                     /note="SINE; putative"
                     /rpt_family="MIR"
repeat_region        92286..92564
                     /note="putative"
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                     /rpt_family="MLT1B"
gene                 92716..93256
                     /gene="TCRBVrelic"
                     /note="This gene was formerly called TCRBV34S1"
V_segment            join(92716..92757,92891..93249)
                     /gene="TCRBVrelic"
                     /pseudo
misc_recomb          93250..93256
                     /gene="TCRBVrelic"
                     /note="RSS_heptamer"
misc_recomb          93250..>93256
                     /gene="TCRBVrelic"
                     /note="RSS degraded RSS"
repeat_region        complement(93367..93856)
                     /note="putative"
                     /rpt_family="MLT1D"
repeat_region        95474..95569
                     /note="LINE; putative"
                     /rpt_family="L1MA9"
repeat_region        96203..96341
                     /note="putative"
                     /rpt_family="Alu"
gene                 96540..97226
                     /gene="TCRBV14S1"
                     /note="proposed new name: TCRBV27S1"
CDS                  join(96715..96763,96893..>97187)
                     /gene="TCRBV14S1"
                     /codon_start=1
                     /product="V_segment translation product"
                     /protein_id="AAC80210.1"
                     /db_xref="GI:1552512"
V_segment            join(96715..96763,96893..97187)
                     /gene="TCRBV14S1"
                     /standard_name="TCRBV14S1"
misc_recomb          97188..97194
                     /gene="TCRBV14S1"
                     /note="RSS_heptamer"
misc_recomb          97195..97217
                     /gene="TCRBV14S1"
                     /note="RSS_spacer"
misc_recomb          97218..97226
                     /gene="TCRBV14S1"
                     /note="RSS_nonamer"
misc_feature         98452..98490
                     /standard_name="TCRBV relic (exon 1)"
                     /note="65% similar to TCRBV13 (6) variable gene regions;
                     putative"
misc_feature         98587..98832
                     /standard_name="TCRBV relic (exon 2, partially)"
                     /note="
                     65% similar to TCRBV13 (6) variable gene regions;
                     putative"
repeat_region        99112..99409
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        complement(100211..100434)
                     /note="LINE; putative"
                     /rpt_family="L1MB1; fragment 1"
repeat_region        100435..100516
                     /note="LINE; putative"
```

```
                     /rpt_family="L1MB1; fragment 2"
repeat_region        complement(100722..100775)
                     /note="SINE; putative"
                     /rpt_family="MIR"
repeat_region        100849..100881
                     /note="(GA)16, 88% conserved; putative"
                     /rpt_type=tandem
repeat_region        100928..101208
                     /note="Unclassified; putative"
                     /rpt_family="MER68B; fragment 1"
repeat_region        101209..101572
                     /note="putative"
                     /rpt_family="MLT1B"
repeat_region        101573..101824
                     /note="Unclassified; putative"
                     /rpt_family="MER68B; fragment 2"
gene                 101934..102524
                     /gene="TCRBV3S1"
                     /note="proposed new name: TCRBV28S1"
misc_feature         101934..101949
                     /gene="TCRBV3S1"
                     /note="conserved 16mer; possible promoter"
CDS                  join(102005..102053,102191..>102485)
                     /gene="TCRBV3S1"
                     /codon_start=1
                     /product="V_segment translation product"
                     /protein_id="AAC80211.1"
                     /db_xref="GI:1552513"
V_segment            join(102005..102053,102191..102485)
                     /gene="TCRBV3S1"
                     /standard_name="TCRBV3S1"
variation            102172
                     /gene="TCRBV3S1"
                     /note="cosmid C21: t; cosmid X11: a"
                     /replace="a"
misc_recomb          102486..102492
                     /gene="TCRBV3S1"
                     /note="RSS_heptamer"
misc_recomb          102493..102515
                     /gene="TCRBV3S1"
                     /note="RSS_spacer"
misc_recomb          102516..102524
                     /gene="TCRBV3S1"
                     /note="RSS_nonamer"
repeat_region        103607..103681
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER75; fragment 1"
repeat_region        104132..104296
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER75; fragment 2"
repeat_region        complement(104802..105102)
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        105294..105318
                     /note="(TATT)6; putative"
                     /rpt_type=tandem
repeat_region        105316..105754
                     /note="putative"
                     /rpt_family="LINE1"
repeat_region        106986..107340
```

```
                     /note="putative"
                     /rpt_family="THE1 transposon like element"
repeat_region        complement(107341..108894)
                     /note="MaLR internal sequence; putative"
                     /rpt_family="THE1A; fragment 2"
repeat_region        complement(108895..109242)
                     /note="MaLR retroposon LTR; putative"
                     /rpt_family="THE1A; fragment 3"
variation            109175
                     /note="cosmid C21: g; cosmid X11: a"
                     /replace="a"
variation            109419
                     /note="cosmid C21: a; cosmid X11: g"
                     /replace="g"
variation            109869
                     /note="cosmid C21: g; cosmid X11: c"
                     /replace="c"
variation            109909
                     /note="cosmid C21: g; cosmid X11: a"
                     /replace="a"
variation            110371
                     /note="cosmid C21: g; cosmid X11: a"
                     /replace="a"
variation            111093
                     /note="cosmid C21: a; cosmid X11: c"
                     /replace="c"
variation            111548
                     /note="cosmid C21: c; cosmid X11: t"
                     /replace="t"
variation            111796
                     /note="cosmid C21: c; cosmid X11: t"
                     /replace="t"
repeat_region        112484..113478
                     /note="LINE; putative"
                     /rpt_family="L1M"
misc_feature         112897
                     /note="The 3' breakpoint occurs at basepair 592356,
                     602910, 613477, or 623282 -- identical positions within a
                     homology unit that is tandemly repeated.; translocation
                     breakpoint t(7:9); putative"
repeat_region        113844..114644
                     /note="LINE; putative"
                     /rpt_family="L1"
repeat_region        114645..115417
                     /note="LINE; putative"
                     /rpt_family="L1; fragment 1"
repeat_region        115418..115715
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        115623..117542
                     /note="putative"
                     /rpt_family="LINE 1"
repeat_region        115716..116924
                     /note="LINE; putative"
                     /rpt_family="L1; fragment 2"
repeat_region        116925..117511
                     /note="putative"
                     /rpt_family="LINE1"
repeat_region        117590..117663
                     /note="LINE; putative"
```

```
                     /rpt_family="L1ME2; fragment 1"
repeat_region        117663..118227
                     /note="Unclassified; putative"
                     /rpt_family="MER76"
repeat_region        118237..118298
                     /note="LINE; putative"
                     /rpt_family="L1PA16"
repeat_region        118388..118494
                     /note="LINE; putative"
                     /rpt_family="L1ME2; fragment 2"
repeat_region        118503..119265
                     /note="LINE; putative"
                     /rpt_family="L1ME2; fragment 3"
variation            118532
                     /note="cosmid X11: t; cosmid X6A: c"
                     /replace="c"
variation            118644..118645
                     /note="cosmid X11: ca; cosmid X6A: a"
                     /replace="a"
repeat_region        119290..119510
                     /note="LINE; putative"
                     /rpt_family="MER42C"
variation            119956
                     /note="cosmid X11: g; cosmid X6A: a"
                     /replace="a"
repeat_region        120605..120728
                     /note="SINE; putative"
                     /rpt_family="MIR"
variation            120817
                     /note="cosmid X11: g; cosmid X6A: a"
                     /replace="a"
variation            121134
                     /note="cosmid X11: c; cosmid X6A: a"
                     /replace="a"
variation            121139
                     /note="cosmid X11: g; cosmid X6A: a"
                     /replace="a"
gene                 121623..122275
                     /gene="TCRBV4S1A1T"
                     /note="proposed new name: TCRBV29S1"
CDS                  join(121623..121656,121936..>122236)
                     /gene="TCRBV4S1A1T"
                     /codon_start=1
                     /product="V_segment translation product"
                     /protein_id="AAC80212.1"
                     /db_xref="GI:1552514"
V_segment            join(121623..121656,121936..122236)
                     /gene="TCRBV4S1A1T"
                     /standard_name="TCRBV4S1"
variation            121703
                     /gene="TCRBV4S1A1T"
                     /note="cosmid X11: g; cosmid X6A: ggtggaaag"
                     /replace="ggtggaaag"
misc_recomb          122237..122243
                     /gene="TCRBV4S1A1T"
                     /note="RSS_heptamer"
misc_recomb          122244..122266
                     /gene="TCRBV4S1A1T"
                     /note="RSS_spacer"
misc_recomb          122267..122275
```

```
                     /gene="TCRBV4S1A1T"
                     /note="RSS_nonamer"
repeat_region        122525..123132
                     /note="endogenous retrovirus, internal sequence; putative"
                     /rpt_family="similarity to MLT2 internal sequence"
variation            122796
                     /note="cosmid X11: t; cosmid X6A: c"
                     /replace="c"
repeat_region        123174..123641
                     /note="Unclassified; putative"
                     /rpt_family="MER70A"
repeat_region        123778..124158
                     /note="endogenous retrovirus, internal sequence; putative"
                     /rpt_family="similarity to MLT2 internal sequence"
repeat_region        125169..125320
                     /note="SINE; putative"
                     /rpt_family="MIR"
variation            125706
                     /note="cosmid X11: t; cosmid X6A: c"
                     /replace="c"
repeat_region        125909..126230
                     /note="LINE; putative"
                     /rpt_family="MIR2"
variation            125993..125998
                     /note="cosmid X11: atatta; cosmid X6A: a"
                     /replace="a"
variation            126318
                     /note="cosmid X11: g; cosmid X6A: c"
                     /replace="c"
repeat_region        126420..126527
                     /note="LINE; putative"
                     /rpt_family="L1MA5A"
variation            127274
                     /note="cosmid X11: c; cosmid X6A: t"
                     /replace="t"
variation            127284
                     /note="cosmid X11: g; cosmid X6A: c"
                     /replace="c"
variation            127471
                     /note="cosmid X11: a; cosmid X6A: c"
                     /replace="c"
variation            127520
                     /note="cosmid X11: a; cosmid X6A: aga"
                     /replace="aga"
repeat_region        complement(127845..127912)
                     /note="putative"
                     /rpt_family="MIR"
repeat_region        127847..127950
                     /note="putative"
                     /rpt_family="MIR1"
repeat_region        127988..128228
                     /note="putative"
                     /rpt_family="MIR"
variation            128051
                     /note="cosmid X11: t; cosmid X6A: g"
                     /replace="g"
misc_feature         128064..138860
                     /standard_name="duplication_unit"
                     /note="trypsinogen duplication unit 1; putative"
variation            128144
```

```
                    /note="cosmid X11: a; cosmid X6A: g"
                    /replace="g"
variation           128148..128149
                    /note="cosmid X11: ac; cosmid X6A: ca"
                    /replace="ca"
variation           128362
                    /note="cosmid X11: g; cosmid X6A: t"
                    /replace="t"
variation           129021
                    /note="cosmid X11: g; cosmid X6A: a"
                    /replace="a"
variation           129032
                    /note="cosmid X11: c; cosmid X6A: t"
                    /replace="t"
repeat_region       129127..129206
                    /note="SINE; putative"
                    /rpt_family="MIR"
variation           129447
                    /note="cosmid X11: g; cosmid X6A: a"
                    /replace="a"
variation           130422
                    /note="cosmid X11: c; cosmid X6A: t"
                    /replace="t"
variation           130626
                    /note="cosmid X11: c; cosmid X6A: a"
                    /replace="a"
gene                130830..134365
                    /gene="TRY4"
                    /note="This gene is known as the cationic trypsinogen."
mRNA                join(<130830..130869,131900..132059,133119..133372,
                    133776..133912,134213..>134365)
                    /gene="TRY4"
                    /note="putative"
CDS                 join(130830..130869,131900..132059,133119..133372,
                    133776..133912,134213..134365)
                    /gene="TRY4"
                    /note="This gene is also known as cationic trypsinogen"
                    /codon_start=1
                    /product="trypsinogen A"
                    /protein_id="AAC80207.1"
                    /db_xref="GI:1552515"
exon                <130830..130869
                    /gene="TRY4"
                    /note="putative"
                    /number=1
intron              130870..131899
                    /gene="TRY4"
                    /note="putative; does not fit consensus"
                    /number=1
variation           131168
                    /gene="TRY4"
                    /note="cosmid X11: g; cosmid X6A: a"
                    /replace="a"
repeat_region       complement(131209..131366)
                    /note="DNA transposon fossil; putative"
                    /rpt_family="MER5B"
exon                131900..132059
                    /gene="TRY4"
                    /note="putative"
                    /number=2
```

```
intron          132060..133118
                /gene="TRY4"
                /note="putative"
                /number=2
repeat_region   132347..132518
                /note="DNA transposon fossil; putative"
                /rpt_family="MER5B"
repeat_region   complement(132533..132677)
                /note="DNA transposon fossil; putative"
                /rpt_family="MER5A"
variation       132784
                /gene="TRY4"
                /note="cosmid X11: t; cosmid X6A: c"
                /replace="c"
exon            133119..133372
                /gene="TRY4"
                /note="putative"
                /number=3
intron          133373..133775
                /gene="TRY4"
                /note="putative"
                /number=3
exon            133776..133912
                /gene="TRY4"
                /note="putative"
                /number=4
variation       133807
                /gene="TRY4"
                /note="cosmid X11: c; cosmid X6A: t"
                /replace="t"
intron          133913..134212
                /gene="TRY4"
                /note="putative"
                /number=4
exon            134213..>134365
                /gene="TRY4"
                /note="putative"
                /number=5
variation       134359
                /gene="TRY4"
                /note="cosmid X11: c; cosmid X6A: t"
                /replace="t"
polyA_signal    134396..134401
                /note="putative"
variation       134772
                /note="cosmid X11: c; cosmid X6A: t"
                /replace="t"
variation       135030
                /note="cosmid X11: c; cosmid X6A: g"
                /replace="g"
variation       135042
                /note="cosmid X11: g; cosmid X6A: a"
                /replace="a"
variation       136066
                /note="cosmid X11: g; cosmid X6A: a"
                /replace="a"
variation       136167
                /note="cosmid X11: g; cosmid X6A: c"
                /replace="c"
variation       136575
```

```
                    /note="cosmid X11: c; cosmid X6A: t"
                    /replace="t"
repeat_region       complement(136750..136997)
                    /note="putative"
                    /rpt_family="Alu"
variation           136801
                    /note="cosmid X11: t; cosmid X6A: c"
                    /replace="c"
misc_feature        136844..147739
                    /note="Overlap with a duplication in cosmid 1H, from CGM1
                    haplotype A"
repeat_region       137021..137732
                    /note="putative"
                    /rpt_family="LINE1"
variation           137212
                    /note="cosmid X11: t; cosmids X6A and 1H: c"
                    /replace="c"
variation           137415
                    /note="cosmid X11: a; cosmids X6A and 1H: c"
                    /replace="c"
variation           137417
                    /note="cosmid X11: c; cosmids X6A and 1H: t"
                    /replace="t"
variation           137689..137692
                    /note="compared to cosmid X6A"
                    /replace="t"
variation           137689..137692
                    /note="compared to cosmid X11 and cosmid 1H"
                    /replace="ttttt"
variation           137762
                    /note="cosmid X11: a; cosmids X6A and 1H: g"
                    /replace="g"
repeat_region       137867..138147
                    /note="putative"
                    /rpt_family="Alu"
misc_feature        137905..183339
                    /note="Overlap with cosmid 1H, from CGM1 haplotype A.
                    This cosmid contains a 20 kb deletion of trypsinogen
                    duplication units 3 and 4.  It also contains a 9.8 kb
                    duplication of part of trypsinogen duplications units 1
                    and 2."
repeat_region       137964..138112
                    /note="putative"
                    /rpt_family="Alu"
variation           137985
                    /note="cosmid X11: a; cosmids X6A and 1H; g"
                    /replace="g"
variation           138011
                    /note="cosmid X11: t; cosmids X6A and 1H: c"
                    /replace="c"
variation           138128
                    /note="cosmids X11 and X6A: a; cosmid 1H: aa"
                    /replace="aa"
variation           138183
                    /note="cosmids X11 and 1H: g; cosmid X6A: a"
                    /replace="a"
variation           138187
                    /note="cosmids X11 and 1H: g; cosmid X6A: tg"
                    /replace="tg"
variation           138677
```

```
                    /note="cosmid X6A: t; cosmid 1H: g"
                    /replace="g"
repeat_region       138841..139015
                    /note="SINE; putative"
                    /rpt_family="MIR"
misc_feature        138858..149438
                    /standard_name="duplication_unit"
                    /note="trypsinogen duplication unit 2; putative"
variation           138944..138945
                    /note="cosmid X6A: ca; cosmid 1H: a"
                    /replace="a"
variation           139839
                    /note="cosmid X6A: t' cosmid 1H: g"
                    /replace="g"
repeat_region       139922..140001
                    /note="SINE; putative"
                    /rpt_family="MIR"
variation           140839
                    /note="cosmid X6A: a; cosmid 1H: g"
                    /replace="g"
variation           141073
                    /note="cosmid X6A: t; comsid 1H: c.  RFLP: HindIII"
                    /replace="c"
variation           141190
                    /note="cosmid X6A: c; cosmid 1H: a"
                    /replace="a"
variation           141239
                    /note="cosmid X6A: t; cosmid 1H: g"
                    /replace="g"
gene                141760..145325
                    /gene="TRY5"
                    /note="Exon 1 splice donor violates consensus.  This gene
                    contains two internal stop codons"
                    /pseudo
mRNA                join(<141760..141799,142819..142978,144038..144291,
                    144699..144835,145137..145287)
                    /gene="TRY5"
                    /note="putative"
                    /pseudo
CDS                 join(141760..141799,142819..142978,144038..144291,
                    144699..144835,145137..145287)
                    /gene="TRY5"
                    /note="putative"
                    /pseudo
                    /codon_start=1
                    /product="trypsinogen B"
exon                <141760..141799
                    /gene="TRY5"
                    /note="putative"
                    /number=1
                    /pseudo
variation           141797
                    /gene="TRY5"
                    /note="cosmid X6A: a; cosmid 1H: c"
                    /replace="c"
intron              141800..142818
                    /gene="TRY5"
                    /note="putative"
                    /number=1
                    /pseudo
```

```
misc_difference 141932
                /gene="TRY5"
                /note="sequence of cosmid X6A should be cc, not c"
                /replace="cc"
repeat_region   complement(142135..142286)
                /note="DNA transposon fossil; putative"
                /rpt_family="MER5B"
variation       142706
                /gene="TRY5"
                /note="cosmid X6A: c; cosmid 1H: t"
                /replace="t"
exon            142819..142978
                /gene="TRY5"
                /note="putative"
                /number=2
                /pseudo
intron          142979..144037
                /gene="TRY5"
                /note="putative"
                /number=2
                /pseudo
repeat_region   143267..143436
                /note="DNA transposon fossil; putative"
                /rpt_family="MER5B"
repeat_region   complement(143452..143596)
                /note="DNA transposon fossil; putative"
                /rpt_family="MER5A"
exon            144038..144291
                /gene="TRY5"
                /note="putative"
                /number=3
                /pseudo
variation       144069
                /gene="TRY5"
                /note="cosmid X6A: a; cosmid 1H: g"
                /replace="g"
intron          144292..144698
                /gene="TRY5"
                /note="putative"
                /number=3
                /pseudo
exon            144699..144835
                /gene="TRY5"
                /note="putative"
                /number=4
                /pseudo
intron          144836..145136
                /gene="TRY5"
                /note="putative"
                /number=4
                /pseudo
exon            145137..145287
                /gene="TRY5"
                /note="putative"
                /number=5
                /pseudo
repeat_region   145202..145267
                /note="LINE; putative"
                /rpt_family="L1PA2"
polyA_signal    145320..145325
```

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                     /gene="TRY5"
                     /note="putative"
variation            146341
                     /note="cosmid X6A: t; cosmid 1H: c"
                     /replace="c"
repeat_region        complement(147646..147890)
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        complement(147915..148615)
                     /note="LINE; putative"
                     /rpt_family="L1PA16"
variation            148119
                     /note="cosmid X6A: g; cosmid 1H: a"
                     /replace="a"
repeat_region        complement(148197..148556)
                     /note="putative"
                     /rpt_family="LINE 1"
repeat_region        149419..149513
                     /note="SINE; putative"
                     /rpt_family="MIR"
variation            149436..169811
                     /note="Cosmid 1H, CGM1 haplotype A, is deleted for this
                     region"
misc_feature         149436..160008
                     /standard_name="duplication_unit"
                     /note="trypsinogen duplication unit 3; putative"
repeat_region        150491..150570
                     /note="SINE; putative"
                     /rpt_family="MIR"
gene                 152252..155896
                     /gene="TRY6"
mRNA                 join(152252..152375,153405..153564,154623..154876,
                     155272..155408,155708..>155860)
                     /gene="TRY6"
                     /note="putative"
exon                 152252..152375
                     /gene="TRY6"
                     /note="putative"
                     /number=1
CDS                  join(152336..152375,153405..153564,154623..154876,
                     155272..155408,155708..155860)
                     /gene="TRY6"
                     /note="putative"
                     /codon_start=1
                     /product="trypsinogen C"
                     /protein_id="AAC80208.1"
                     /db_xref="GI:1552516"
intron               152376..153404
                     /gene="TRY6"
                     /note="putative"
                     /number=1
repeat_region        complement(152711..152873)
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER5B"
exon                 153405..153564
                     /gene="TRY6"
                     /note="putative"
                     /number=2
intron               153565..154622
                     /gene="TRY6"
```

```
                     /note="putative"
                     /number=2
repeat_region        153851..154020
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER5B"
repeat_region        complement(154036..154181)
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER5A"
exon                 154623..154876
                     /gene="TRY6"
                     /note="putative"
                     /number=3
intron               154877..155271
                     /gene="TRY6"
                     /note="putative"
                     /number=3
exon                 155272..155408
                     /gene="TRY6"
                     /note="putative"
                     /number=4
intron               155409..155707
                     /gene="TRY6"
                     /note="putative"
                     /number=4
exon                 155708..>155860
                     /gene="TRY6"
                     /note="putative"
polyA_signal         155891..155896
                     /gene="TRY6"
                     /note="putative"
repeat_region        158228..158457
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        complement(158492..159189)
                     /note="LINE; putative"
                     /rpt_family="L1PA16"
repeat_region        complement(158773..159123)
                     /note="putative"
                     /rpt_family="LINE 1"
repeat_region        160000..160082
                     /note="SINE; putative"
                     /rpt_family="MIR"
misc_feature         160006..169814
                     /standard_name="duplication_unit"
                     /note="trypsinogen duplication unit 4; putative"
repeat_region        161056..161135
                     /note="SINE; putative"
                     /rpt_family="MIR"
gene                 162421..165990
                     /gene="TRY7"
                     /pseudo
mRNA                 join(<162421..162460,163497..163656,164715..164968,
                     165369..165505,165803..>165952)
                     /gene="TRY7"
                     /note="putative"
                     /pseudo
CDS                  join(162421..162460,163497..163656,164715..164968,
                     165369..165505,165803..165952)
                     /gene="TRY7"
                     /note="putative"
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                    /pseudo
                    /codon_start=1
                    /product="trypsinogen D"
exon                <162421..162460
                    /gene="TRY7"
                    /note="putative"
                    /number=1
                    /pseudo
intron              162461..163496
                    /gene="TRY7"
                    /note="putative"
                    /number=1
                    /pseudo
repeat_region       complement(162803..162960)
                    /note="DNA transposon fossil; putative"
                    /rpt_family="MER5B"
exon                163497..163656
                    /gene="TRY7"
                    /note="putative"
                    /number=2
                    /pseudo
intron              163657..164714
                    /gene="TRY7"
                    /note="no splice donor; putative; does not fit consensus"
                    /number=2
                    /pseudo
repeat_region       163944..164114
                    /note="DNA transposon fossil; putative"
                    /rpt_family="MER5B"
repeat_region       complement(164129..164273)
                    /note="DNA transposon fossil; putative"
                    /rpt_family="MER5A"
exon                164715..164968
                    /gene="TRY7"
                    /note="putative"
                    /number=3
                    /pseudo
intron              164969..165368
                    /gene="TRY7"
                    /note="putative"
                    /number=3
                    /pseudo
exon                165369..165505
                    /gene="TRY7"
                    /note="putative"
                    /number=4
                    /pseudo
intron              165506..165802
                    /gene="TRY7"
                    /note="putative"
                    /number=4
                    /pseudo
exon                165803..>165952
                    /gene="TRY7"
                    /note="putative"
                    /number=5
                    /pseudo
polyA_signal        165985..165990
                    /gene="TRY7"
                    /note="putative"
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repeat_region   complement(168335..168583)
                /note="putative"
                /rpt_family="Alu"
repeat_region   complement(168600..168991)
                /note="LINE; putative"
                /rpt_family="L1PA16"
repeat_region   complement(168613..168887)
                /note="putative"
                /rpt_family="LINE 1"
repeat_region   169795..169933
                /note="SINE; putative"
                /rpt_family="MIR"
misc_feature    169812..180402
                /standard_name="duplication_unit"
                /note="trypsinogen duplication unit 5; putative"
variation       169941..169942
                /note="cosmid CBG1: cc; cosmid 1H: c"
                /replace="c"
variation       170020
                /note="cosmid CBG1: g; cosmid 1H: c"
                /replace="c"
variation       170344
                /note="cosmid CBG1: c; cosmid 1H; a"
                /replace="a"
variation       170426
                /note="cosmid CBG1: t; cosmid 1H: c"
                /replace="c"
repeat_region   170873..170952
                /note="SINE; putative"
                /rpt_family="MIR"
variation       172303
                /note="cosmid CBG1: g; cosmid 1H: a"
                /replace="a"
gene            172704..176267
                /gene="TRY8"
                /note="This gene is known as the anionic trypsinogen"
mRNA            join(<172704..172743,173772..173931,174989..175242,
                175642..175778,176079..>176267)
                /gene="TRY8"
                /note="putative"
CDS             join(172704..172743,173772..173931,174989..175242,
                175642..175778,176079..176231)
                /gene="TRY8"
                /note="This gene is also known as anionic trypsinogen"
                /codon_start=1
                /product="trypsinogen E"
                /protein_id="AAC80209.1"
                /db_xref="GI:1552517"
exon            <172704..172743
                /gene="TRY8"
                /note="putative"
                /number=1
intron          172744..173771
                /gene="TRY8"
                /note="putative"
                /number=1
repeat_region   complement(173082..173239)
                /note="DNA transposon fossil; putative"
                /rpt_family="MER5B"
variation       173093
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                     /gene="TRY8"
                     /note="cosmid CBG1: a; cosmid 1H: g"
                     /replace="g"
variation            173099
                     /gene="TRY8"
                     /note="cosmid CBG1: c; cosmid 1H: t"
                     /replace="t"
variation            173102..173104
                     /gene="TRY8"
                     /note="cosmid CBG1: aaa; cosmid 1H; a"
                     /replace="a"
variation            173117
                     /gene="TRY8"
                     /note="cosmid CBG1: c; cosmid 1H: a"
                     /replace="a"
variation            173121
                     /gene="TRY8"
                     /note="cosmid CBG1: g; cosmid 1H: c"
                     /replace="c"
exon                 173772..173931
                     /gene="TRY8"
                     /note="putative"
                     /number=2
intron               173932..174988
                     /gene="TRY8"
                     /note="putative"
                     /number=2
repeat_region        174218..174389
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER5B"
repeat_region        complement(174404..174548)
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER5A"
variation            174858
                     /gene="TRY8"
                     /note="cosmid CG1: g; cosmid 1H: a"
                     /replace="a"
exon                 174989..175242
                     /gene="TRY8"
                     /note="putative"
                     /number=3
variation            175058
                     /gene="TRY8"
                     /note="cosmid CG1: a; cosmid 1H: g"
                     /replace="g"
intron               175243..175641
                     /gene="TRY8"
                     /note="putative"
                     /number=3
exon                 175642..175778
                     /gene="TRY8"
                     /note="putative"
                     /number=4
intron               175779..176078
                     /gene="TRY8"
                     /note="putative"
                     /number=4
variation            176070
                     /gene="TRY8"
                     /note="cosmid CG1: c; cosmid 1H: t"
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                /replace="t"
exon            176079..>176267
                /gene="TRY8"
                /note="putative"
                /number=5
polyA_signal    176262..176267
                /gene="TRY8"
                /note="putative"
variation       176377
                /note="cosmid CBG1: c; cosmid 1H: a"
                /replace="a"
variation       176709
                /note="cosmid CBG1: t; cosmid 1H: c"
                /replace="c"
variation       176747
                /note="cosmid CBG1: a; cosmid 1H: g"
                /replace="g"
variation       177030
                /note="cosmid CBG1: g; cosmid 1H: a"
                /replace="a"
variation       177747
                /note="cosmid CBG1: t; cosmid 1H: c"
                /replace="c"
variation       177891..177892
                /note="cosmid CBG1: tt; cosmid 1H; t"
                /replace="t"
variation       177957
                /note="cosmid CBG1: a; cosmid 1H: g"
                /replace="g"
variation       178135
                /note="cosmid CBG1: t; cosmid 1H: c"
                /replace="c"
variation       178144
                /note="cosmid CBG1: gt; cosmid 1H: ta"
                /replace="ta"
variation       178502
                /note="cosmid CBG1: c; cosmid 1H: a"
                /replace="a"
repeat_region   complement(178601..178891)
                /note="putative"
                /rpt_family="Alu"
variation       178645
                /note="cosmid CBG1: c; cosmid 1H: t"
                /replace="t"
repeat_region   178730..178780
                /note="(TTTA)13; putative"
                /rpt_type=tandem
variation       178730..178738
                /note="cosmid CBG1: tttatttat; cosmid 1H: t"
                /replace="t"
repeat_region   complement(178916..179615)
                /note="LINE; putative"
                /rpt_family="L1PA16"
repeat_region   complement(179129..179605)
                /note="putative"
                /rpt_family="LINE 1"
misc_difference 179448..179450
                /note="cosmid CBG1 sequence should be gg, not ggg"
                /replace="gg"
misc_difference 179587
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                     /note="cosmid CBG1 sequence should be tt, not t"
                     /replace="tt"
variation            179825
                     /note="cosmid CBG1: a; cosmid 1H: c"
                     /replace="c"
variation            180083
                     /note="cosmid CBG1: a; cosmid 1H: g"
                     /replace="g"
variation            180363
                     /note="cosmid CBG1: a; cosmid 1H: g"
                     /replace="g"
variation            181268
                     /note="cosmid CBG1: g; cosmid 1H: a"
                     /replace="a"
variation            181667
                     /note="cosmid CBG1: t; cosmid 1H: c"
                     /replace="c"
variation            181758
                     /note="cosmid CBG1: c; cosmid 1H: t"
                     /replace="t"
repeat_region        complement(181885..182013)
                     /note="SINE; putative"
                     /rpt_family="MIR"
variation            182068
                     /note="cosmid CBG1: a; cosmid 1H: g"
                     /replace="g"
variation            182610
                     /note="cosmid CBG1: g; cosmid 1H: a"
                     /replace="a"
repeat_region        182777..182902
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER5A; fragment 1"
repeat_region        complement(182911..183201)
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        183203..183261
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER5A; fragment 2"
repeat_region        183295..183362
                     /note="endogenous retroviral LTR; putative"
                     /rpt_family="LTR8; fragment 1"
variation            183327
                     /note="cosmid CBG1: g; cosmid 1H: a"
                     /replace="a"
repeat_region        complement(183365..183684)
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        183685..184343
                     /note="putative"
                     /rpt_family="HUMERSP1B"
repeat_region        complement(184383..184683)
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        complement(184734..184815)
                     /note="SINE; putative"
                     /rpt_family="MIR"
repeat_region        complement(185608..185907)
                     /note="putative"
                     /rpt_family="Alu"
repeat_region        complement(186570..186910)
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                     /note="SINE; putative"
                     /rpt_family="AluSx"
repeat_region        complement(187037..187215)
                     /note="LINE; putative"
                     /rpt_family="L1M4"
gene                 187917..187995
                     /gene="TCRBD1"
misc_recomb          187917..187944
                     /gene="TCRBD1"
                     /note="RSS; nonamer is at 5' end"
misc_recomb          187917..187925
                     /gene="TCRBD1"
                     /note="RSS_nonamer"
misc_recomb          187926..187937
                     /gene="TCRBD1"
                     /note="RSS_spacer"
misc_recomb          187938..187944
                     /gene="TCRBD1"
                     /note="RSS_heptamer"
D_segment            187945..187956
                     /gene="TCRBD1"
                     /note="putative"
misc_recomb          187957..187995
                     /gene="TCRBD1"
                     /note="RSS heptamer is at 5' end"
misc_recomb          187957..187963
                     /gene="TCRBD1"
                     /note="RSS_heptamer"
misc_recomb          187964..187986
                     /gene="TCRBD1"
                     /note="RSS_spacer"
misc_recomb          187987..187995
                     /gene="TCRBD1"
                     /note="RSS_nonamer"
gene                 188584..188659
                     /gene="TCRBJ1S1"
misc_recomb          188584..188611
                     /gene="TCRBJ1S1"
                     /note="RSS nonamer is at 5' end"
misc_recomb          188584..188592
                     /gene="TCRBJ1S1"
                     /note="RSS_nonamer"
misc_recomb          188593..188604
                     /gene="TCRBJ1S1"
                     /note="RSS_spacer"
misc_recomb          188605..188611
                     /gene="TCRBJ1S1"
                     /note="RSS_heptamer"
J_segment            188612..188659
                     /gene="TCRBJ1S1"
                     /note="putative"
gene                 188721..188796
                     /gene="TCRBJ1S2"
misc_recomb          188721..188748
                     /gene="TCRBJ1S2"
                     /note="RSS nonamer is at 5' end"
misc_recomb          188721..188729
                     /gene="TCRBJ1S2"
                     /note="RSS_nonamer"
misc_recomb          188730..188741
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                     /gene="TCRBJ1S2"
                     /note="RSS_spacer"
     misc_recomb     188742..188748
                     /gene="TCRBJ1S2"
                     /note="RSS_heptamer"
     J_segment       188749..188796
                     /gene="TCRBJ1S2"
                     /note="putative"
     gene            189334..189411
                     /gene="TCRBJ1S3"
     misc_recomb     189334..189361
                     /gene="TCRBJ1S3"
                     /note="RSS nonamer is at 5' end"
     misc_recomb     189334..189342
                     /gene="TCRBJ1S3"
                     /note="RSS_nonamer"
     misc_recomb     189343..189354
                     /gene="TCRBJ1S3"
                     /note="RSS_spacer"
     misc_recomb     189355..189361
                     /gene="TCRBJ1S3"
                     /note="RSS_heptamer"
     J_segment       189362..189411
                     /gene="TCRBJ1S3"
                     /note="putative"
     gene            189929..190007
                     /gene="TCRBJ1S4"
     misc_recomb     189929..189956
                     /gene="TCRBJ1S4"
                     /note="RSS nonamer is at 5' end"
     misc_recomb     189929..189937
                     /gene="TCRBJ1S4"
                     /note="RSS_nonamer"
     misc_recomb     189938..189949
                     /gene="TCRBJ1S4"
                     /note="RSS_spacer"
     misc_recomb     189950..189956
                     /gene="TCRBJ1S4"
                     /note="RSS_heptamer"
     J_segment       189957..190007
                     /gene="TCRBJ1S4"
                     /note="putative"
     gene            190202..190279
                     /gene="TCRBJ1S5"
     misc_recomb     190202..190229
                     /gene="TCRBJ1S5"
                     /note="RSS nonamer is at 5' end"
     misc_recomb     190202..190210
                     /gene="TCRBJ1S5"
                     /note="RSS_nonamer"
     misc_recomb     190211..190222
                     /gene="TCRBJ1S5"
                     /note="RSS_spacer"
     misc_recomb     190223..190229
                     /gene="TCRBJ1S5"
                     /note="RSS_heptamer"
     J_segment       190230..190279
                     /gene="TCRBJ1S5"
                     /note="putative"
     gene            190692..190772
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misc_recomb         190692..190719
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misc_recomb         190692..190700
                    /gene="TCRBJ1S6"
                    /note="RSS_nonamer"
misc_recomb         190701..190712
                    /gene="TCRBJ1S6"
                    /note="RSS_spacer"
misc_recomb         190713..190719
                    /gene="TCRBJ1S6"
                    /note="RSS_heptamer"
J_segment           190720..190772
                    /gene="TCRBJ1S6"
                    /note="putative"
repeat_region       complement(191526..191819)
                    /note="putative"
                    /rpt_family="Alu"
gene                193426..195080
                    /gene="TCRBC1"
CDS                 join(<193426..193812,194254..194271,194424..194530,
                    194853..194873)
                    /gene="TCRBC1"
                    /note="C_region translation product"
                    /codon_start=3
                    /product="V_segment translation product"
                    /protein_id="AAC80213.1"
                    /db_xref="GI:1552518"
C_region            join(193426..193812,194254..194271,194424..194530,
                    194853..194873)
                    /gene="TCRBC1"
                    /note="putative"
exon                193426..193812
                    /gene="TCRBC1"
                    /note="putative"
                    /number=1
intron              193813..194253
                    /gene="TCRBC1"
                    /note="putative"
                    /number=1
exon                194254..194271
                    /gene="TCRBC1"
                    /note="putative"
                    /number=2
intron              194272..194423
                    /gene="TCRBC1"
                    /note="putative"
                    /number=2
exon                194424..194530
                    /gene="TCRBC1"
                    /note="putative"
                    /number=3
intron              194531..194852
                    /gene="TCRBC1"
                    /note="putative"
                    /number=3
exon                194853..>195080
                    /gene="TCRBC1"
                    /note="putative"
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                     /number=4
polyA_signal         195075..195080
                     /gene="TCRBC1"
                     /note="putative"
gene                 197409..197491
                     /gene="TCRBD2"
misc_recomb          197409..197436
                     /gene="TCRBD2"
                     /note="RSS nonamer is at 5' end"
misc_recomb          197409..197417
                     /gene="TCRBD2"
                     /note="RSS_nonamer"
misc_recomb          197418..197429
                     /gene="TCRBD2"
                     /note="RSS_spacer"
misc_recomb          197430..197436
                     /gene="TCRBD2"
                     /note="RSS_heptamer"
D_segment            197437..197448
                     /gene="TCRBD2"
                     /note="putative"
misc_recomb          197453..197491
                     /gene="TCRBD2"
                     /note="RSS heptamer is at 5' end"
misc_recomb          197453..197459
                     /gene="TCRBD2"
                     /note="RSS_heptamer"
misc_recomb          197460..197482
                     /gene="TCRBD2"
                     /note="RSS_spacer"
misc_recomb          197483..197491
                     /gene="TCRBD2"
                     /note="RSS_nonamer"
repeat_region        complement(197708..197835)
                     /note="SINE; putative"
                     /rpt_family="MIR"
gene                 198069..198146
                     /gene="TCRBJ2S1"
misc_recomb          198069..198096
                     /gene="TCRBJ2S1"
                     /note="RSS nonamer is at 5' end"
misc_recomb          198069..198077
                     /gene="TCRBJ2S1"
                     /note="RSS_nonamer"
misc_recomb          198078..198089
                     /gene="TCRBJ2S1"
                     /note="RSS_spacer"
misc_recomb          198090..198096
                     /gene="TCRBJ2S1"
                     /note="RSS_heptamer"
J_segment            198097..198146
                     /gene="TCRBJ2S1"
                     /note="putative"
gene                 198264..198342
                     /gene="TCRBJ2S2"
misc_recomb          198264..198291
                     /gene="TCRBJ2S2"
                     /note="RSS nonamer is at 5' end"
misc_recomb          198264..198272
                     /gene="TCRBJ2S2"
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                     /note="RSS_nonamer"
misc_recomb          198273..198284
                     /gene="TCRBJ2S2"
                     /note="RSS_spacer"
misc_recomb          198285..198291
                     /gene="TCRBJ2S2"
                     /note="RSS_heptamer"
J_segment            198292..198342
                     /gene="TCRBJ2S2"
                     /note="putative"
gene                 198551..198627
                     /gene="TCRBJ2S3"
misc_recomb          198551..198578
                     /gene="TCRBJ2S3"
                     /note="RSS nonamer is at 5' end"
misc_recomb          198551..198559
                     /gene="TCRBJ2S3"
                     /note="RSS_nonamer"
misc_recomb          198560..198571
                     /gene="TCRBJ2S3"
                     /note="RSS_spacer"
misc_recomb          198572..198578
                     /gene="TCRBJ2S3"
                     /note="RSS_heptamer"
J_segment            198579..198627
                     /gene="TCRBJ2S3"
                     /note="putative"
gene                 198702..198779
                     /gene="TCRBJ2S4"
misc_recomb          198702..198729
                     /gene="TCRBJ2S4"
                     /note="RSS nonamer is at 5' end"
misc_recomb          198702..198710
                     /gene="TCRBJ2S4"
                     /note="RSS_nonamer"
misc_recomb          198711..198722
                     /gene="TCRBJ2S4"
                     /note="RSS_spacer"
misc_recomb          198723..198729
                     /gene="TCRBJ2S4"
                     /note="RSS_heptamer"
J_segment            198730..198779
                     /gene="TCRBJ2S4"
                     /note="putative"
gene                 198823..198898
                     /gene="TCRBJ2S5"
misc_recomb          198823..198850
                     /gene="TCRBJ2S5"
                     /note="RSS nonamer is at 5' end"
misc_recomb          198823..198831
                     /gene="TCRBJ2S5"
                     /note="RSS_nonamer"
misc_recomb          198832..198843
                     /gene="TCRBJ2S5"
                     /note="RSS_spacer"
misc_recomb          198844..198850
                     /gene="TCRBJ2S5"
                     /note="RSS_heptamer"
J_segment            198851..198898
                     /gene="TCRBJ2S5"
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                     /note="putative"
gene                 198943..199023
                     /gene="TCRBJ2S6"
misc_recomb          198943..198970
                     /gene="TCRBJ2S6"
                     /note="RSS nonamer is at 5' end"
misc_recomb          198943..198951
                     /gene="TCRBJ2S6"
                     /note="RSS_nonamer"
misc_recomb          198952..198963
                     /gene="TCRBJ2S6"
                     /note="RSS_spacer"
misc_recomb          198964..198970
                     /gene="TCRBJ2S6"
                     /note="RSS_heptamer"
J_segment            198971..199023
                     /gene="TCRBJ2S6"
                     /note="putative"
gene                 199160..199234
                     /gene="TCRBJ2S7"
misc_recomb          199160..199187
                     /gene="TCRBJ2S7"
                     /note="RSS nonamer is at 5' end"
misc_recomb          199160..199168
                     /gene="TCRBJ2S7"
                     /note="RSS_nonamer"
misc_recomb          199169..199180
                     /gene="TCRBJ2S7"
                     /note="RSS_spacer"
misc_recomb          199181..199187
                     /gene="TCRBJ2S7"
                     /note="RSS_heptamer"
J_segment            199188..199234
                     /gene="TCRBJ2S7"
                     /note="putative"
repeat_region        200331..200491
                     /note="putative"
                     /rpt_family="MIR"
repeat_region        200732..200954
                     /note="(AGAAGCTCACTAGTACACACACTTACACC)9, 29mer  93%
                     conserved; putative"
                     /rpt_type=tandem
repeat_region        201382..201521
                     /note="DNA transposon fossil; putative"
                     /rpt_family="MER45"
gene                 202772..204451
                     /gene="TCRBC2"
CDS                  join(<202772..203158,203675..203692,203836..203942,
                     204234..204260)
                     /gene="TCRBC2"
                     /note="C_region translation product"
                     /codon_start=3
                     /product="V_segment translation product"
                     /protein_id="AAC80214.1"
                     /db_xref="GI:1552519"
C_region             join(202772..203158,203675..203692,203836..203942,
                     204234..204260)
                     /gene="TCRBC2"
                     /note="putative"
exon                 202772..203158
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                     /note="putative"
                     /number=1
intron               203159..203674
                     /gene="TCRBC2"
                     /note="putative"
                     /number=1
exon                 203675..203692
                     /gene="TCRBC2"
                     /note="putative"
                     /number=2
intron               203693..203835
                     /gene="TCRBC2"
                     /note="putative"
                     /number=2
exon                 203836..203942
                     /gene="TCRBC2"
                     /note="putative"
                     /number=3
intron               203943..204233
                     /gene="TCRBC2"
                     /note="putative"
                     /number=3
exon                 204234..>204451
                     /gene="TCRBC2"
                     /note="putative"
                     /number=4
polyA_signal         204446..204451
                     /gene="TCRBC2"
                     /note="putative"
repeat_region        complement(205372..205494)
                     /note="SINE; putative"
                     /rpt_family="MIR"
repeat_region        complement(205687..206131)
                     /note="putative"
                     /rpt_family="MLT1B"
repeat_region        209230..209399
                     /note="SINE; putative"
                     /rpt_family="MIR"
repeat_region        210121..210192
                     /note="SINE; putative"
                     /rpt_family="MIR"
repeat_region        complement(212621..212747)
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                     /rpt_family="MIR"
repeat_region        complement(213389..213689)
                     /note="putative"
                     /rpt_family="Alu"
gene                 complement(214278..215017)
                     /gene="TCRBV20S1A1N2"
                     /note="proposed new name: TCRBV30S1"
misc_recomb          complement(214278..214316)
                     /gene="TCRBV20S1A1N2"
                     /note="RSS nonamer is at 5' end"
misc_recomb          214278..214286
                     /note="RSS_nonamer"
misc_recomb          214287..214309
                     /note="RSS_spacer"
misc_recomb          214310..214316
                     /note="RSS_heptamer"
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CDS             complement(join(<214317..214608,214975..215017))
                /gene="TCRBV20S1A1N2"
                /codon_start=1
                /product="V_segment translation product"
                /protein_id="AAC80215.1"
                /db_xref="GI:1552520"
V_segment       complement(join(214317..214608,214975..215017))
                /gene="TCRBV20S1A1N2"
                /standard_name="TCRBV20S1"
repeat_region   215724..215829
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                /rpt_family="MIR"
repeat_region   215893..215924
                /note="(T)32; putative"
                /rpt_type=tandem
repeat_region   217870..218384
                /note="putative"
                /rpt_family="MLT1D"
repeat_region   complement(219117..219570)
                /note="putative"
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repeat_region   219573..220863
                /note="LINE; putative"
                /rpt_family="L191"
repeat_region   221157..222776
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                /note="DNA transposon fossil; putative"
                /rpt_family="MER63"
repeat_region   223111..224502
                /note="LINE; putative"
                /rpt_family="L1M"
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                /rpt_family="MER77"
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                /note="LINE; putative"
                /rpt_family="L1M; fragment 2"
repeat_region   227925..228253
                /note="LINE; putative"
                /rpt_family="L1ME1; fragment 1"
repeat_region   228318..228610
                /note="putative"
                /rpt_family="Alu"
repeat_region   228611..228984
                /note="LINE; putative"
                /rpt_family="L1ME1; fragment 2"
repeat_region   228980..229437
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```
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                     /rpt_family="Alu"
     repeat_region   231527..231833
                     /note="LINE; putative"
                     /rpt_family="L1ME; fragment 2"
ORIGIN
//
```

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NCBI | NLM | NIH

Apr 10 2008 15:51:27

APPENDIX 4

Blast 2 Sequences results

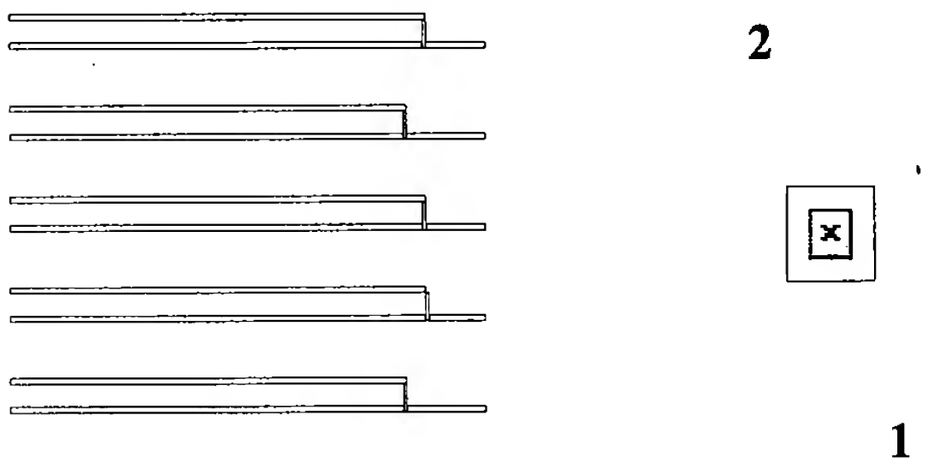
PubMed Entrez BLAST OMIM Taxonomy Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.18 [Mar-02-2008]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2
x_dropoff: 0 expect: 10.000(wordsize: 11 Filter ☑ View option Standard
Masking character option X for protein, n for nucleotide Masking color option Black
☐ Show CDS translation Align

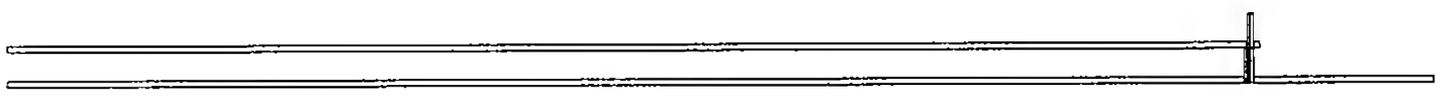
Sequence 1: lcl|1
Length = 862 (1 .. 862)

Sequence 2: gi|1552511|Human germline T-cell receptor beta chain TCRBV17S1A1T, TCRBV2S1, TCRBV10S1P, TCRBV29S1P, TCRBV19S1P, TCRBV15S1, TCRBV11S1A1T, HVB relic, TCRBV28S1P, TCRBV34S1, TCRBV14S1, TCRBV3S1, TCRBV4S1A1T, TRY4, TRY5, TRY6, TRY7, TRY8, TCRBD1, TCRBJ1S1, TCRBJ1S2, TCRBJ1S3, TCRBJ1S4, TCRBJ1S5, TCRBJ1S6, TCRBC1, TCRBD2, TCRBJ2S1, TCRBJ2S2, TCRBJ2S3, TCRBJ2S4, TCRBJ2S5, TCRBJ2S6, TCRBJ2S7, TCRBC2, TCRBV20S1A1N2 genes from bases 452324 to 684973 (section 3 of 3)
Length = 232650 (1 .. 232650)



NOTE:Bitscore and expect value are calculated based on the size of the nr database.

NOTE:If protein translation is reversed, please repeat the search with reverse strand of the query sequence.



```
 Score =  735 bits (382),  Expect = 0.0
 Identities = 386/388 (99%), Gaps = 0/388 (0%)
 Strand=Plus/Plus

Query  1       GAGGACCTGAAAAACGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAG  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  202771  GAGGACCTGAAAAACGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAG  202830

Query  61      ATCTCCCACACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTACCCCGACCAC  120
```

```
                ||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  202831   ATCTCCCACACCCAAAAGGCCACACTGGTATGCCTGGCCACAGGCTTCTACCCCGACCAC  202890

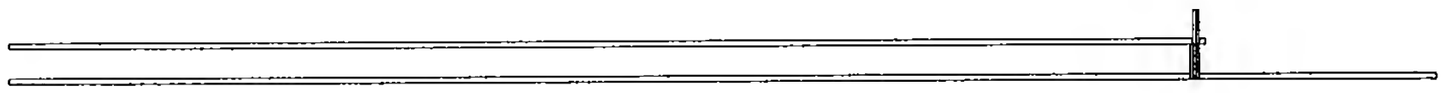
Query  121      GTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCG  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  202891   GTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCG  202950

Query  181      CAGCCCCTCAAGGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTG  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  202951   CAGCCCCTCAAGGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTG  203010

Query  241      AGGGTCTCGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTC  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  203011   AGGGTCTCGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTC  203070

Query  301      TACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATC  360
                ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  203071   TACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATC  203130

Query  361      GTCAGCGCCGAGGCCTGGGGTAGAGCAG  388
                ||||||||||||||||||||||||||||
Sbjct  203131   GTCAGCGCCGAGGCCTGGGGTAGAGCAG  203158
```



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 Strand=Plus/Plus

Query  1        GAGGACCTGAAAAACGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAG  60
                |||||||||||  ||  ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  193425   GAGGACCTGAACAAGGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAG  193484

Query  61       ATCTCCCACACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTACCCCGACCAC  120
                ||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  193485   ATCTCCCACACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTTCCCCGACCAC  193544

Query  121      GTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCG  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  193545   GTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCG  193604

Query  181      CAGCCCCTCAAGGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTG  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  193605   CAGCCCCTCAAGGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTG  193664

Query  241      AGGGTCTCGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTC  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  193665   AGGGTCTCGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTC  193724

Query  301      TACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATC  360
                ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  193725   TACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATC  193784

Query  361      GTCAGCGCCGAGGCCTGGGGTAGAGCAG  388
                ||||||||||||||||||||||||||||
Sbjct  193785   GTCAGCGCCGAGGCCTGGGGTAGAGCAG  193812
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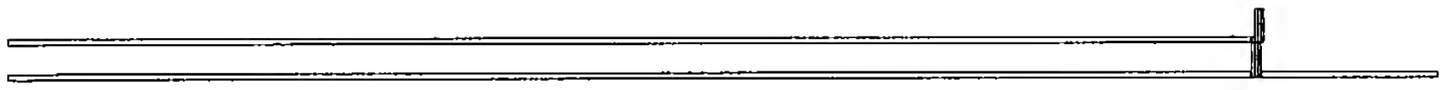

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 Strand=Plus/Plus

Query  386     CAGACTGTGGCTTCACCTCCGGTAAGTGAGTCTCTCCTTTTTCTCTCTATCTTTCGCCGT  445
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  203672  CAGACTGTGGCTTCACCTCCGGTAAGTGAGTCTCTCCTTTTTCTCTCTATCTTTCGCCGT  203731

Query  446     CTCTGCTCTCGAACCAGGGCATGGAGAATCCACGGACACAGGGGCGTGAGGGAGGCCAGA  505
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  203732  CTCTGCTCTCGAACCAGGGCATGGAGAATCCACGGACACAGGGGCGTGAGGGAGGCCAGA  203791

Query  506     GCCACCTGTGCACAGGTACCTACATGCTCTGTTCTTGTCAACAGAGTCTTACCAGCAAGG  565
               |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  203792  GCCACCTGTGCACAGGTGCCTACATGCTCTGTTCTTGTCAACAGAGTCTTACCAGCAAGG  203851

Query  566     GGTCCTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAGGCCACCTTGTATGCCGT  625
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  203852  GGTCCTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAGGCCACCTTGTATGCCGT  203911

Query  626     GCTGGTCAGTGCCCTCGTGCTGATGGCCATGGT  658
               ||||||||||||||||||||||||||||||||
Sbjct  203912  GCTGGTCAGTGCCCTCGTGCTGATGGCCATGGT  203944
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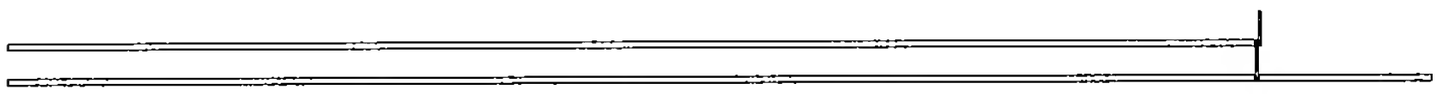

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 Identities = 207/207 (100%), Gaps = 0/207 (0%)
 Strand=Plus/Plus

Query  656     GGTCAAGAGAAAGGATTCCAGAGGCTAGCTCCAAAACCATCCCAGGTCATTCTTCATCCT  715
               |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204233  GGTCAAGAGAAAGGATTCCAGAGGCTAGCTCCAAAACCATCCCAGGTCATTCTTCATCCT  204292

Query  716     CACCCAGGATTCTCCTGTACCTGCTCCCAATCTGTGTTCCTAAAAGTGATTCTCACTCTG  775
               |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204293  CACCCAGGATTCTCCTGTACCTGCTCCCAATCTGTGTTCCTAAAAGTGATTCTCACTCTG  204352

Query  776     CTTCTCATCTCCTACTTACATGAATACTTCTCTCTTTTTTCTGTTTCCCTGAAGATTGAG  835
               |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  204353  CTTCTCATCTCCTACTTACATGAATACTTCTCTCTTTTTTCTGTTTCCCTGAAGATTGAG  204412

Query  836     CTCCCAACCCCCAAGTACGAAATAGGC  862
               |||||||||||||||||||||||||||
Sbjct  204413  CTCCCAACCCCCAAGTACGAAATAGGC  204439
```

```
 Score =  202 bits (105),  Expect = 2e-48
 Identities = 228/282 (80%), Gaps = 9/282 (3%)
 Strand=Plus/Plus

Query  386     CAGACTGTGGCTTCACCTCCGGTAAGTGAGTCTCTCCTTTTTCTCTCTATCTTTCGCCGT  445
               ||||||||||||| ||||| ||||||| || |  ||||||| |||||  ||| ||   ||
Sbjct  194251  CAGACTGTGGCTTTACCTCGGGTAAGTAAGCCCTTCCTTTTCCTCTCCCTCTCTCATGGT  194310

Query  446     CTCTGCTCTCGAACCAGGGCATGGAGAATCCACGGACACAGGGGCGTG-AGGGAGG----  500
                  ||  || |||||| |||||| ||||  ||| ||||| || | ||| |||| ||
Sbjct  194311  TCTTGACCTAGAACCAAGGCATGAAGAACTCACAGACACTGGAGGGTGGAGGGTGGGAGA  194370

Query  501     --CCAGAGCCACCTGTGCACAGGTACCTACATGCTCTG--TTCTTGTCAACAGAGTCTTA  556
                 ||||||| |||||||||||||||| || ||  ||   | | || ||||||  ||| ||
Sbjct  194371  GACCAGAGCTACCTGTGCACAGGTACCCACCTGTCCTTCCTCCGTGCCAACAGTGTCCTA  194430

Query  557     CCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAGGCCACCTT  616
               |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||| |
Sbjct  194431  CCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGCCACCCT  194490

Query  617     GTATGCCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCATGGT  658
               |||||| |||||||||| |||| ||| ||||||||||||
Sbjct  194491  GTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGT  194532


CPU time:      0.05 user secs.       0.04 sys. secs          0.09 total secs.
```

APPENDIX 5

Appendix 5. Translation Map of the Bandman Sequence (SEQ ID NO: 130)

The nucleotide assignment of the Bandman sequence is given under the amino acid translation. The sequences identical between the Bandman sequence and SEQ ID NO:67 and the translated polypeptide SEQ ID NO:51, are bolded.
Note that a cluster of stop codons at the 5' end of the Cß2 region makes the polynucleotide untranslatable.

```
cggaggactcctggttctgggtgctgggagancgatggggctctcagcggtgggaaggacc 60
                                  M  G  L  S  A  V  G  R  T   9
                                  < ----------------------
cgagctgagtctgggacagcagagcgggcagcaccggttttgtcctgggcctccaggct  120
 R  A  E  S  G  T  A  E  R  A  A  P  V  F  V  L  G  L  Q  A   29
-----------    5' intronic upstream of Jß2.3      ---------
Gtgagcacagatacgcagtattttggcccaggcacccggctgacagtgctcggtaagcgg  180
 V  S  T  D  T  Q  Y  F  G  P  G  T  R  L  T  V  L  G  K  R   49
 --- >< -----        Jß2.3 exon              ----- >< -------
Gggctcccgctgaagcccgggaactggggaggggcgccccgggacgccgggggcgtcgc  240
 G  L  P  L  K  P  G  N  W  G  G  G  A  P  G  R  R  G  R  R   69
----------------   intron      --------------------------
Agggccagtttctgtgccgcgtctcggggctgtgagccaaaaacattcagtacttcggcg  300
 R  A  S  F  C  A  A  S  R  G  C  E  P  K  T  F  S  T  S  A   89
--------- >< -----------      Jß2.4 exon    ----------------
Ccgggacccggctctcagtgctggaggacctgaaaaacgtgttcccacccgaggtcgctg  360
 P  G  P  G  S  Q  C  W  R  T Stop K  T  C  S  H  P  R  S  L  109
---------------------- > <-- Cß2 (up to the end of the sequence)
Tgtttgagccatcagaagcagagatctcccacacccaaaaggccacactggtgtgcctgg
 C  L  S  H  Q  K  Q  R  S  P  T  P  K  R  P  H  W  C  A  W
Ccacaggcttctaccccgaccacgtggagctgagctggtgggtgaatgggaaggaggtgc
 P  Q  A  S  T  P  T  T  W  S Stop A  G  G Stop M  G  R  R  C
acagtggggtcagcacagacccgcagcccctcaaggagcagcccgccctcaatgactcca
 T  V  G  S  A  Q  T  R  S  P  S  R  S  S  P  P  S  M  T  P
gatactgcctgagcagccgcctgagggtctcggccaccttctggcagaaccccccgcaacc
 D  T  A Stop A  A  A Stop G  S  R  P  P  S  G  R  T  P  A  T
acttccgctgtcaagtccagttctacgggctctcggagaatgacgagtggacccaggata
 T  S  A  V  K  S  S  S  T  G  S  R  R  M  T  S  G  P  R  I
gggccaaacctgtcacccagatcgtcagcgccgaggcctggggtagagcagactgtggct
 G  P  N  L  S  P  R  S  S  A  P  R  P  G  V  E  Q  T  V  A
tcacctccggtaagtgagtctcctttttctctctatctttcgccgtctctgctctcga
 S  P  P  V  S  E  S  L  L  F  L  S  I  F  R  R  L  C  S  R
accagggcatggagaatccacggacacaggggcgtgagggaggccagagccacctgtgca
 T  R  A  W  R  I  H  G  H  R  G  V  R  E  A  R  A  T  C  A
caggtacctacatgctctgttcttgtcaacagagtcttaccagcaaggggtcctgtctgc
 Q  V  P  T  C  S  V  L  V  N  R  V  L  P  A  R  G  P  V  C
caccatcctctatgagatcttgctagggaaggccaccttgtatgccgtgctggtcagtgc
 H  H  P  L Stop D  L  A  R  E  G  H  L  V  C  R  A  G  Q  C
cctcgtgctgatggccatggtcaagagaaaggattccagaggctagctccaaaaccatcc
 P  R  A  D  G  H  G  Q  E  K  G  F  Q  R  L  A  P  K  P  S
caggtcattcttcatcctcacccaggattctcctgtacctgctcccaatctgtgttccta
 Q  V  I  L  H  P  H  P  G  F  S  C  T  C  S  Q  S  V  F  L
aaagtgattctcactctgcttctcatctcctacttacatgaatacttctctcttttttct
 K  V  I  L  T  L  L  L  I  S  Y  L  H  E  Y  F  S  L  F  S
gtttccctgaagattgagctcccaacccccaagtacgaaataggc
 V  S  L  K  I  E  L  P  T  P  K  Y  E  I  G
```